derived from a single individual, nor even from only one of the several sachets of artificial diet on which the original Y5 population was being cultured. And he made only 2 gels contrasting the 2 populations. There is, therefore, no evidence that these extra bands were ever present in the original population of the Y5 clone, or if they were, that they were not confined to one or a few aberrant individuals from which the sub-culture on artificial diet (but not that on potato plants) was derived.

There could be many other explanations for the appearance of these extra bands in only 1 of these 2 sub-cultures, and for their subsequent complete disappearance. But my repeated failure to find any trace of these extra bands in gels made from aphids of the same Y5 clone, and the failure of the original authors to demonstrate the presence of these bands before subjecting the sub-cultures to different diets, precludes any claims being made that diet has an effect on the pattern and number of the esterase bands of *M. persicae.*

1 I thank Professor H.F. van Emden for the opportunity to work in his laboratory and Josephine Pemberton and Barry Tranter for their guidance, help and friendship throughout my apprenticeship in acrylamide-gel-electrophoresis.

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Purification of diphtheria toxin by chromatography on Cibacron Blue-Sepharose

G. Antoni, M. Bigio, G. Borri, M.C. Casagli and P. Neri

ISVT Sclavo Research Center, via Fiorentina 1, I-53100 Siena (Italy), December 13, 1982

Summary. Diphtheria toxin binds to Cibacron Blue-Agarose and may be eluted by increasing the ionic strength of the elution buffer. Experiments using difference spectroscopy showed that the interaction between toxin and dye is ionic rather than hydrophobic, and therefore it is of a different nature with respect to that usually found in nucleotide-requiring enzymes.

Diphtheria toxin inhibits protein synthesis in mammalian cells, catalyzing ADP-ribosylation of elongation factor 2[1]. The toxin exerts its toxic activity after formation of a 1:1 noncovalent complex with NAD[1,2]. Exploiting this interaction, the toxin has been purified by affinity chromatography on NAD-Sepharose[3].

Enzymes which interact with NAD generally also bind the dye Cibacron Blue F3GA, and may be purified by chromatography on a column of Cibacron Blue-Sepharose[4]. The interaction with the dye has been adopted as a probe for the protein supersecondary structure called the 'dinucleotide fold'[4], although the binding is not completely specific, and proteins devoid of the dinucleotide fold may also interact with the dye[5]. It was therefore of interest to examine whether there was also an interaction with Cibacron Blue in the case of diphtheria toxin, which would in some way reflect the interaction with NAD.

We performed our experiments using a crude broth culture of *C. diphtheriae,* grown on a Lingood medium. After removal of the bacteria by centrifugation, the supernatant was sterilized by filtration and dialyzed against 0.02 M phosphate buffer pH 6.9. The solution was then chromatographed on a column of Blue Sepharose CL-6B (Pharmacia, Sweden), giving the elution profile shown in figure 1. The 1st peak of UV-absorbing material was obtained by eluting the column with 0.02 M phosphate buffer, pH 6.9. The buffer was then changed to one containing 2M NaCl, which gave a 2nd peak containing most of the immunochemical activity. The combined fractions of the 2nd peak showed a specific immunochemical activity of about

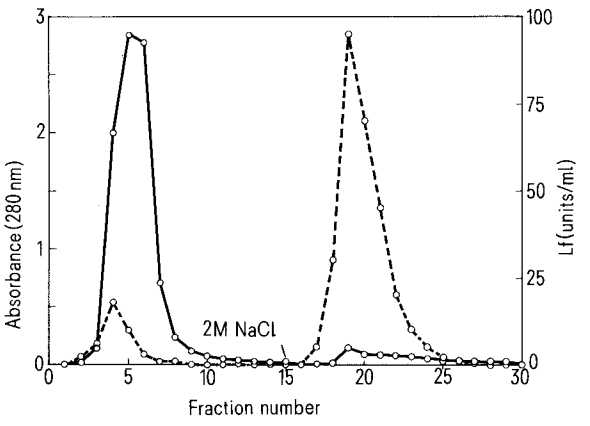

Figure 1. Chromatography of crude diphtheria toxin on a column of Cibacron Blue-Sepharose (1.6×20 cm). Volume of the sample: 30 ml (200 Lf/ml). Volume of the fractions: 10 ml. Flow rate: 70 ml/h. The protein content of the fractions was estimated by measuring the absorbance at 280 nm. The immunochemical activity (dotted line) was determined by rocket immunoelectrophoresis[6] against a specific antiserum, using a reference preparation of toxin, 75 Lf/ml, as standard.

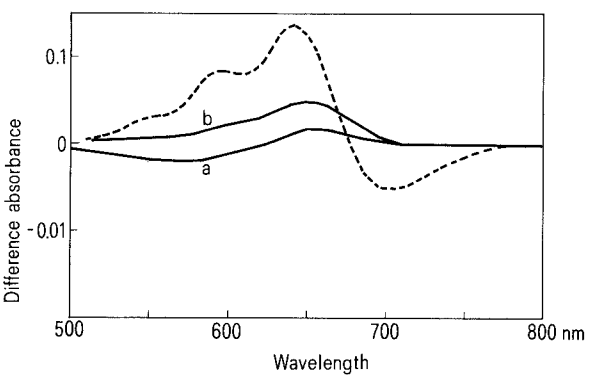

Figure 2. Difference spectrum of Cibacron Blue with diphtheria toxin (highly purified reference sample). The sample cuvette contained 15 μM toxin and Cibacron Blue at concentrations: a) 135 μM; b) 260 μM. The reference cuvette contained the same amount of dye as the sample cuvette. The dotted line presents the spectrum obtained using 15 μM human serum albumin in place of diphtheria toxin and 135 μM Cibacron Blue.

450 Lf/mg protein. Both intact and nicked toxin[7] showed similar behavior.

In order to investigate the mechanism of the interaction between toxin and dye, we also performed experiments using difference spectroscopy, measuring the spectral shift produced by the protein when it binds the free dye in solution. Figure 2 shows that the addition of diphtheria toxin to Cibacron Blue produces a very weak difference spectrum, that cannot be ascribed to a hydrophobic interaction. For comparison, the figure also shows the spectrum produced in the same conditions by a mixture of Cibacron Blue and human serum albumin[8] which presents the typical shape resulting from a hydrophobic interaction[5].

From the results of our experiments we may conclude that the interaction of diphtheria toxin with Cibacron Blue takes place on a region of the protein molecule which presents structural characteristics different from those found in albumin or in nucleotide-requiring enzymes.

The binding of the toxin to the immobilized dye is therefore likely to be ionic rather than hydrophobic; the high degree of specificity of this interaction could eventually be explained by a favorable spatial arrangement of positive charges in the protein molecule, which would facilitate an ionic interaction with the negatively charged sulfonate groups of the dye. Moreover, it is important to bear in mind that the chromatography of the crude broth culture of *C. diphtheriae* on Cibacron Blue-Sepharose produced a toxin of high specific immunologic activity, and therefore it could be applied to purify this protein.

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A histofluorescent procedure for identifying marijuana cannabinoids[1]

A. Bruni, I. Barni Comparini[2] and E. Menziani Andreoli[3]

Institute of Botany, University of Ferrara, Corso Porta Mare 2, I-44100 Ferrara (Italy), December 28, 1982

Summary. A rapid and reliable fluorescence procedure is described as a test for the microscopical identification of the glandular hairs of *Cannabis sativa*. The proposed method, designated as the IFIM test (induced fluorescence identification for marijuana test), is based on the induction of a red fluorescence in cannabinoids by a hot clearing solution. The results, compared to those obtained by the classical RIM test, offer the possibility of more satisfactory identification of cannabis, hashish or marijuana in suspected samples.

Several histochemical tests for marijuana have previously appeared[4–7], but only 2 microscopic procedures are routinely used in many countries for the legal identification of marijuana: the RIM and the vanillin tests[8,9]. The specificity of these methods has been recently confirmed[10], but some uncertainty in the identification of marijuana has been noted due to the weak intensity of the color response and the lack of clarity in the microscopic preparations. In spite of the high potential of fluorescence microscopy, the application of fluorescence methods in pharmacognosy represents a much-neglected field of research[11–13]. The present study was carried out to explore the feasibility of using induced-fluorescence microscopy for localizing the cannabinoids in marijuana plants. Samples of fresh or dried plant material of *Cannabis sativa* 'fiber cultigen', collected locally, and authentic marijuana plants and resin samples, obtained from police seizures, were processed directly on microscope slides with the following procedures: a) direct mounting in water to observe the autofluorescence that naturally occurs under different excitation wavelengths. Thus, it is possible to detect many cellular components such as chlorophyll, proteins, lipids, lignin, suberin, etc., which emit primary fluorescence that can mislead during the microscopic observation of fresh material; b) observation of fluorescence emission when the specimen is mounted in cold clearing solution composed of chloral hydrate (7.5 g), propylene glycol (1.0 ml) and distilled water sufficient to make 10 ml. This clearing procedure dissolves proteins, plant pigments and other autofluorescent substances which tend to confuse the diagnostic criteria of identification. The autofluorescence that resists cold chloral hydrate treatment is normally due to lipids, phenols[14] and, in the case of *Cannabis*, cannabinoids[15]; c) IFIM test (induced fluorescence identification for marijuana test). This new procedure is based on a 1-min heating of samples on a microburner with 4–5 drops of the above-cited clearing solution; d)

Color responses of cannabinoid standard and capitate gland heads of fiber and drug strains of *Cannabis sativa* to IFIM test and other cannabinoid indicators

Cannabinoid indicators	Cannabinoid standard	Capitate glandular trichomes		Color of reactions
		Fiber	Drug	
IFIM	+ +	–	+ + +	red (fluorescence)
RIM	+ +	–	+ +	red
vanillin/ethanolic H_2SO_4	+	–	+	pink

+ + + Intense reaction; + + moderate reaction; + weak reaction; – no reaction.