

ACCUMULATION OF A SIMPLE PEPTIDE AND  
SOME PAULY-POSITIVE COMPOUNDS IN  
CROWN-GALL TUMORS INDUCED BY  
*AGROBACTERIUM TUMEFACIENS*  
STRAINS IIBV7 AND 181<sup>(1,2)</sup>

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Crown-gall tumors have been shown to accumulate some compounds which are very difficult to detect in normal plants. In case of imino acids, octopine [ $N^2$ -(D-1-carboxy ethyl)-L-arginine] and lysopine [ $N^2$ -(D-1-carboxyethyl)-L-lysine] are accumulated only in tumors induced by certain strains of *A. tumefaciens*; whereas nopaline [ $N^2$ -(1,3-dicarboxypropyl)-L-arginine] is accumulated only in tumors induced by some other strains (Goldmann *et al.*, 1969; Petit *et al.*, 1970). But tumors induced by strain 181 and EU6 contain no detectable octopine or nopaline (Petit *et al.*, 1970; Lippincott *et al.*, 1973; Creaser *et al.*, 1976). In a previous paper we reported that strain B<sub>6</sub> induced crown-gall tumors contain a single peptide fraction and an unknown Paily-positive compound which can not be detected in normal plant tissues (Chang, Lin, and Hu, 1975). Now we extend our analyses of these two compounds to the tumors induced by strain IIBV7 and strain 181.

Bacterial strains were obtained from Dr. J. A. Lippincott's laboratory in Northwestern University, Evanston, Ill. U.S.A. Strain IIBV7 induces tumor which accumulates nopaline; while strain B<sub>6</sub> induces tumor which accumulates octopine (and lysopine). Tumor induced by strain 181 accumulates neither octopine nor nopaline. Culture medium and growth condition of the bacterium were described by Heberlain and Lippincott (1965). Tumors were initiated on tomato seedlings by wounding the stem by pushing a syringe needle through the stem and injecting bacterial culture into the wound. Tumors were harvested about one month later. The extraction procedure and analyses of the two

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special crown-gall metabolites were the same as described earlier (Chang *et al.*, 1975). Briefly, the tumor tissues were extracted with three times their weight of methanol. A Dowex 50 (H<sup>+</sup> form) column was used to obtain the cationic fraction of the extract. The cationic fraction was then fractionated on a SP-Sephadex C-25 column (115×2.5 cm) using 0.2 M ammonium formate buffer pH 3.4 to pH 3.6. Fractions from the column were then analyzed by one-dimensional paper chromatography using a solvent system of butanol-acetic acid-water (4:1:5, v/v/v).

Figure 1 shows the one-dimensional chromatograms of fractions from the SP-Sephadex columns on Whatman 1MM chromatographic paper. The chromatographic papers were sprayed with ninhydrin reagent. Tumors induced by strains IIBV7 and 181 all have the ninhydrin-positive special peptide fraction (No. 5 peptide fraction) at around tube 111, which is present in tumors induced by strain B<sub>6</sub> but is undetectable in normal plant tissues. The accumulation of this peptide fraction seems to be specific for crown-gall tumors. Although we also found a similar appearance of a peptide fraction in the extract of nematode gall (Chang, Lin and Hu, 1975), the R<sub>f</sub> value of that peptide was somewhat higher than that of crown-gall peptide. The patterns of ninhydrin-positive components, obtained after acid hydrolysis, on two dimensional paper chromatograms were also different. As a check for the identity of the type of tumor, chromatograms were also sprayed with diacetyl reagent (Smith, 1960). Nopaline was found only in strain IIBV7 induced tumor. No diacetyl positive spot was visible on chromatogram obtained from strain 181 induced tumor.

Figure 2 shows that strain IIBV7 induced tumor contains the Pauly-positive compound (also around tube 111) that was found in strain B<sub>6</sub> induced tumor. This compound is not found in strain 181 induced tumor, which contains two other crown-gall specific unknown Pauly-positive compounds of its own (eluted around tube 75) (Fig. 3).

The occurrence of the special simple peptide fraction in representatives of the three types of tumors is interesting and it may be noted that animal tumors were also reported to accumulate a special simple peptide (Neish *et al.*, 1961). Strain 181 induced tumor accumulates none of the imino acids nor the crown-gall specific Pauly-positive compound found in tumors induced by strain B<sub>6</sub> and strain IIBV7. It, however, accumulates the special simple peptide fraction and two other unknown Pauly-positive compounds. This phenomenon suggests that accumulation of unusual metabolites is a general characteristic of crown-gall tumor cells.

The origin of the genetic information for the unusual metabolites in crown-gall tumors is unknown; the occurrence of octopine in normal tissues, including

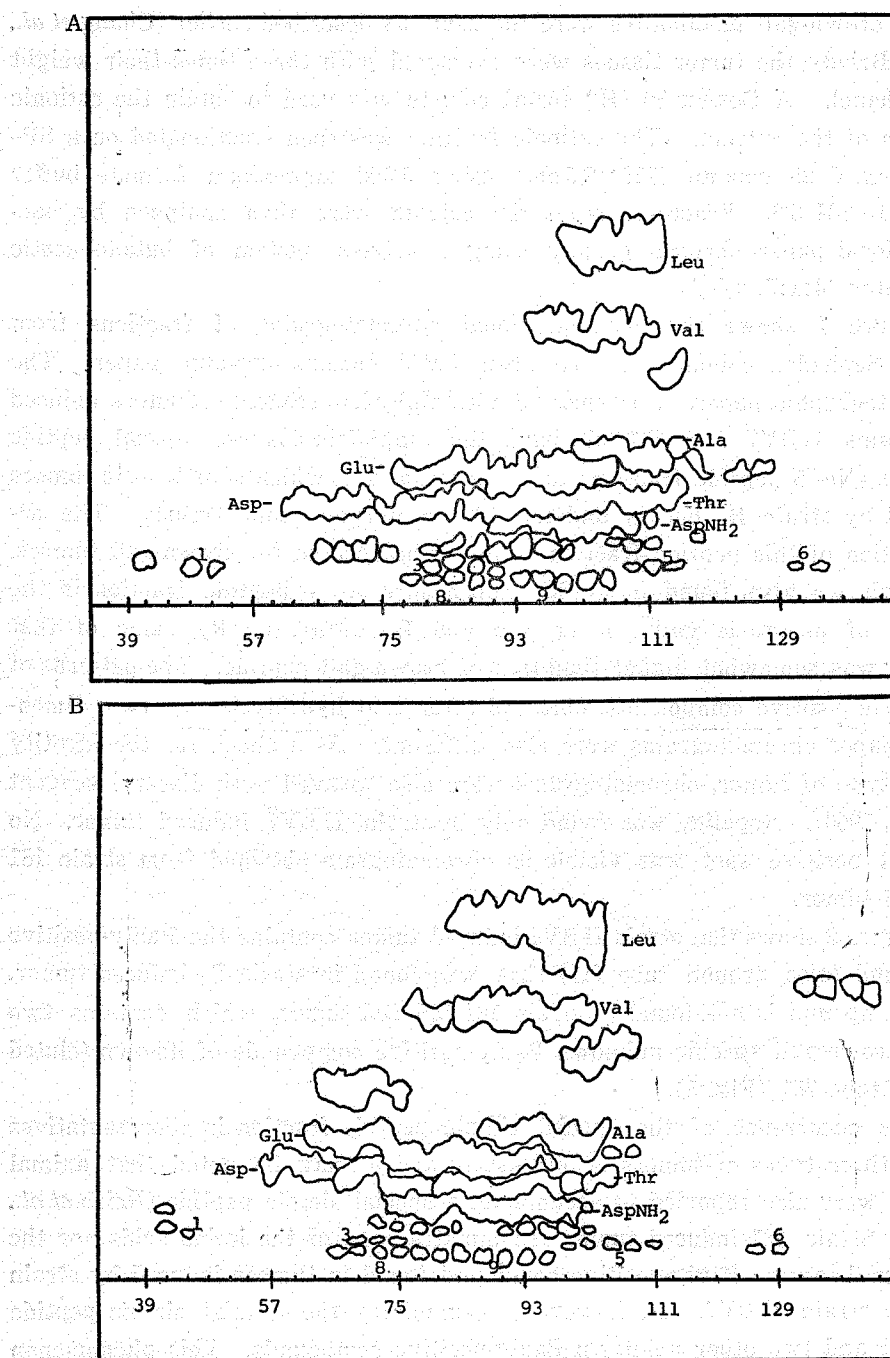


Fig. 1. One-dimensional paper chromatographies of fractions eluted from SP-Sephadex column showing ninhydrin-positive compounds. A: strain IIBV7 induced tomato stem tumor; B: strain 181 induced tomato stem tumor. Only every third fractions were applied to the chromatographic papers. Some amino acids are labeled. Numbers label different sections of peptide spots. No. 5 peptide fraction, which is absent from normal plant extract, is present in both strain IIBV7 and strain 181 induced tumors.

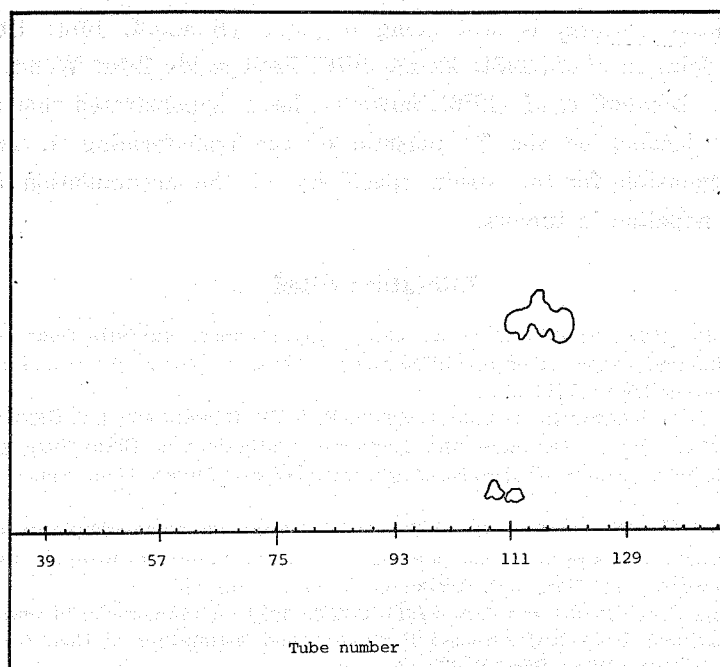


Fig. 2. One-dimensional paper chromatographies of fractions eluted from SP-Sephadex column showing Pauly-positive compounds. Sample fractionated was prepared from extract of strain IIBV7 induced tomato stem tumor. Only every third fractions were applied to the chromatographic papers. The Pauly-positive spots with lower  $R_f$  value (around tube 111) were not found when normal plant extract and strain 181 induced tumor were analyzed.

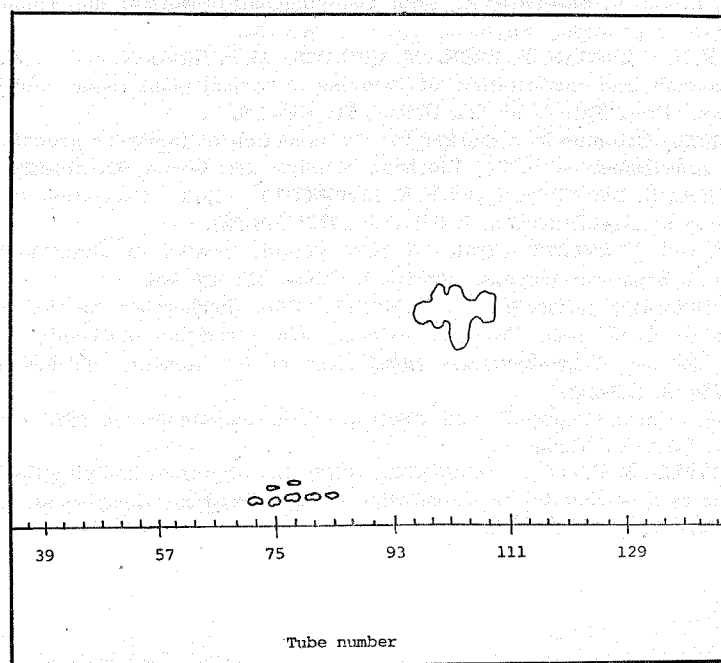


Fig. 3. One-dimensional paper chromatographies of fractions eluted from SP-Sephadex column showing Pauly-positive compounds from strain 181 induced tumor.

habituate tissue culture, is still being disputed (Bomhoff, 1974; Holderbach *et al.*, 1976; Johnson *et al.*, 1974; Kemp, 1976; Petit *et al.*, 1970; Wendt-Gullitelli *et al.*, 1973). Bomhoff *et al.* (1976), however, have demonstrated that a genetic determinant located on the TI plasmid of the transforming *A. tumefaciens* strain is responsible for the strain specificity of the accumulation of either octopine or nopaline in tumors.

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幾種癌腫特殊代謝物在二種  
*A. tumefaciens* 品系 (181 和 IIBV7)  
所引起的癌腫中的分佈

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我們曾報導由 *A. tumefaciens*, B<sub>6</sub> 品系所引起的癌腫中有一種特殊簡單胺和一種特殊 Pauly 氏試劑呈色物。用正常植物做材料都不能測出這兩種化合物的存在。我們再用其他二種品系的細菌引起的蕃茄莖癌腫做材料，比較這兩種化合物和另外兩種癌腫特殊化合物，octopine 和 nopaline，在這三種癌腫中的分佈。由別人的報告我們已經知道，B<sub>6</sub> 品系所引起的癌腫堆積 octopine 但不堆積 nopaline。IIBV7 品系所引起的癌腫堆積 nopaline 但不堆積 octopine。181 品系所引起的癌腫對 octopine 和 nopaline 都沒有堆積現象。我們的結果顯示這三種癌腫中都含有上面提到的特殊簡單胺。而 IIBV7 品系和 B<sub>6</sub> 品系所引起的癌腫一樣也有上面提到的 Pauly 氏試劑呈色物。181 品系所引起的癌腫中雖然沒有這種 Pauly 氏試劑呈色物的堆積，但却含有二種新的未知 Pauly 氏試劑呈色物。這種結果顯示出不正常代謝物的堆積是 *A. tumefaciens* 引起的癌腫的普遍現象。