Proc. Nat. Acad. Sci. USA
Vol. 68, No. 6, pp. 1130–1134, June 1971

Unusual Fragments in the Subunit Structure of Concanavalin A

(gel electrophoresis/molecular weights)

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Communicated March 15, 1971

ABSTRACT Gel electrophoresis in sodium dodecyl sulfate and gel filtration in guanidine·HCl indicate that native concanavalin A contains several molecular species. An intact subunit of molecular weight 27,000 has been purified from this mixture. In addition, three fragments of the intact subunit have been isolated and characterized. A working model of the concanavalin A molecule has been constructed based on pairings of the intact subunit and of subunits consisting of fragments.

Since its first isolation from jack beans by Sumner (1), the protein concanavalin A (Con A) has been shown to possess hemagglutinating activity (2), to precipitate various polysaccharides (3, 4), and to be mitogenic for various animal cells (5, 6). More recently, interest in Con A has been stimulated as a result of the demonstration that variations in binding of this agglutinin may reveal structural changes on membrane surfaces (7, 8) and by the observation that Con A that has been treated with trypsin can restore the growth pattern of transformed fibroblasts to that of normal cells (9).

In the course of experiments to determine the complete amino acid sequence and three-dimensional structure of Con A, we have observed that commercially available Con A, as well as fresh preparations isolated by three different methods (3, 10, 11), each showed four distinct bands on polyacrylamide gel electrophoresis in sodium dodecyl sulfate (SDS). In the present communication, we report the isolation and characterization of the materials corresponding to each of the major bands. These have been identified as an intact subunit and three fragments that account for nearly the entire subunit. Chemical studies on the three fragments have enabled us to establish their order and formulate a chemical model for the native molecule. These assignments have been confirmed by our studies on the cleavage of Con A by cyanogen bromide (manuscript in preparation).

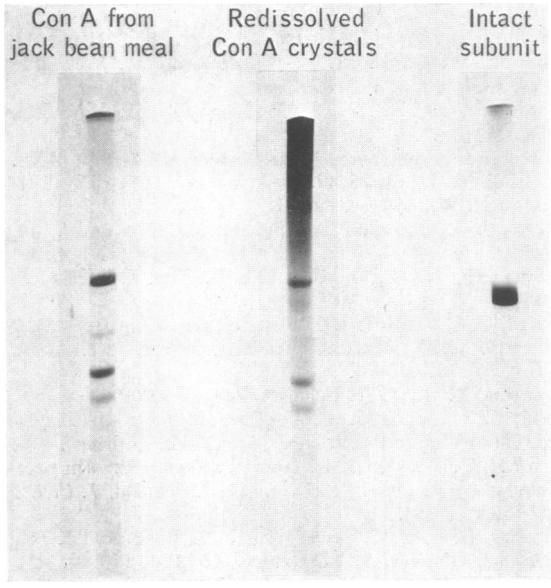

FIG. 1. Polyacrylamide gel electrophoresis in SDS of concanavalin A isolated from jack bean meal.

Abbreviations: Con A, concanavalin A; SDS, sodium dodecyl sulfate.

MATERIALS AND METHODS

Con A was isolated from jack bean meal (Mann, Lot no. 5418; Schwarz-Mann, Lot no. W1376) by the methods of Sumner and Howell (3), Olsen and Liener (10), and Agrawal and Goldstein (11), and from whole jack beans (Sigma, Lot no. 17B-0920) by the method of Agrawal and Goldstein (11). Commercially available Con A samples from Miles Laboratories (3X crystallized, Control no. 15) and Calbiochem (A grade, Lot no. 010229) were used without further purification.

The intact subunit was isolated by ion-exchange chromatography on DEAE-cellulose (Whatman DE-52). Best results

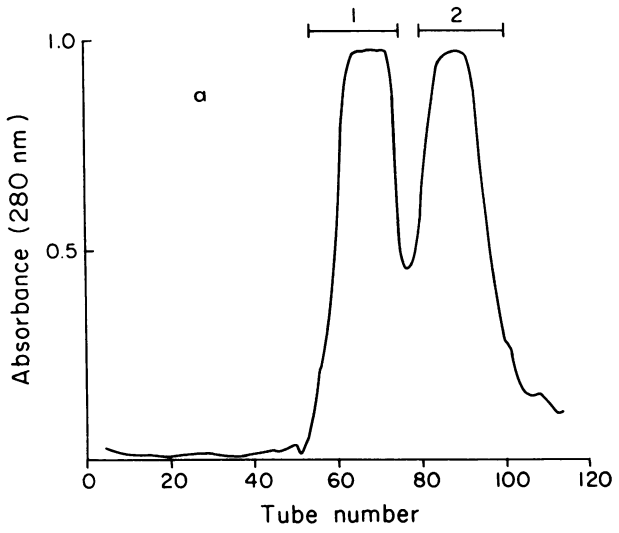

FIG. 2*a*. Gel filtration of concanavalin A on Sephadex G-100 in 6 M guanidine·HCl–1 M propionic acid. Each fraction contained 2.0 ml of effluent. The solid line designates absorbance of effluent fractions at 280 nm.

were obtained when 500 mg of Con A was loaded on a column (3 × 25 cm) equilibrated with 0.02 M Tris–0.03 M KCl, pH 8.0. The fragments were separated from the intact subunit by gel filtration on a column (2.2 × 100 cm) of Sephadex G-100 in 6 M guanidine·HCl–1 M propionic acid. The fragments were then separated from each other by ion-exchange chromatography on a column (1.4 × 18 cm) of DEAE-cellulose in 0.02 M Tris, pH 8.0–8 M deionized urea, eluted with a linear gradient from 0 to 0.5 M KCl.

Con A and its fragments were digested with carboxypeptidase A (Worthington COA 726) in SDS (12). Comparative peptide maps (13, 14) were prepared after digestion of each protein sample with trypsin (Calbiochem, DCC-treated, Lot no. 001449) for 15 hr at 37°C.

Procedures used for amino acid analysis (15), sequence determination by the dansyl-Edman method (16, 17), and polyacrylamide gel electrophoresis in the presence of SDS (18) have been described. Molecular weights of Con A and its fragments were determined by equilibrium sedimentation (19, 20) in 0.1 M Tris–6 M guanidine·HCl (spectrophotometric grade, Heico, Inc.), pH 7.0. Partial specific volumes were calculated from amino acid compositions (21), and were corrected for the presence of guanidine in the solutions (22).

Methods used to assay the mitogenic activity (23) and hemagglutinating activity (24) have been described.

RESULTS

Polyacrylamide gel electrophoresis in SDS of Con A isolated from jack bean meal yielded four bands (Fig. 1). The three major bands corresponded to molecular weights of approximately 27,000, 13,000, and 10,000. There was also a minor component with a molecular weight of 18,000. Con A prepared by several different procedures (3, 10, 11), as well as commercial samples, gave the same pattern. In addition, crystals prepared for high-resolution x-ray diffraction studies (G. N. Reeke and J. W. Becker, unpublished observations) gave a similar gel pattern (Fig. 1).

The heterogeneity of Con A prepared from jack bean meal (11) was confirmed by gel filtration on Sephadex G-100 in a strongly dissociating solvent system (Fig. 2*a*). In contrast, gel filtration of Con A on Bio-Gel P-150 in phosphate buffer (pH 7.4) gave a single component. Fraction *1* (Fig. 2*a*) contained the material having a molecular weight of about 27,000. This accounted for 60% by weight of the total recovered material. Fraction *2* contained a mixture of the lower molecular weight materials (MW 13,000 and 10,000). Peptide maps (14) of

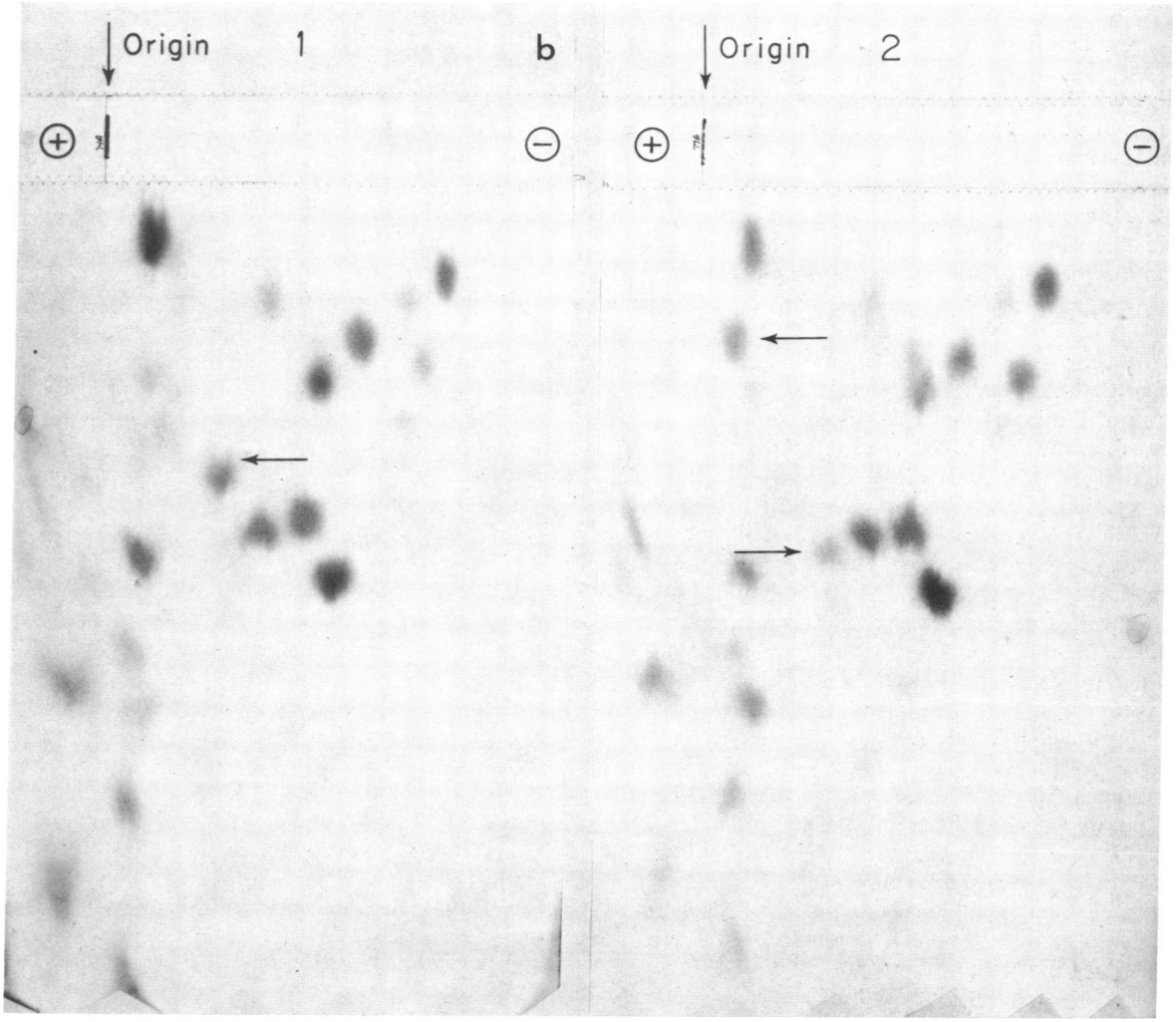

FIG. 2*b*. Peptide maps of tryptic digests of material in fractions *1* and *2* (Fig. 2*a*). Electrophoresis in the first dimension was performed at pH 4.7. Descending chromatography in the second dimension was performed in *n*-butanol–acetic acid–water–pyridine 15:3:12:10. *Horizontal arrows* designate ninhydrin-positive components that differ in the two maps.

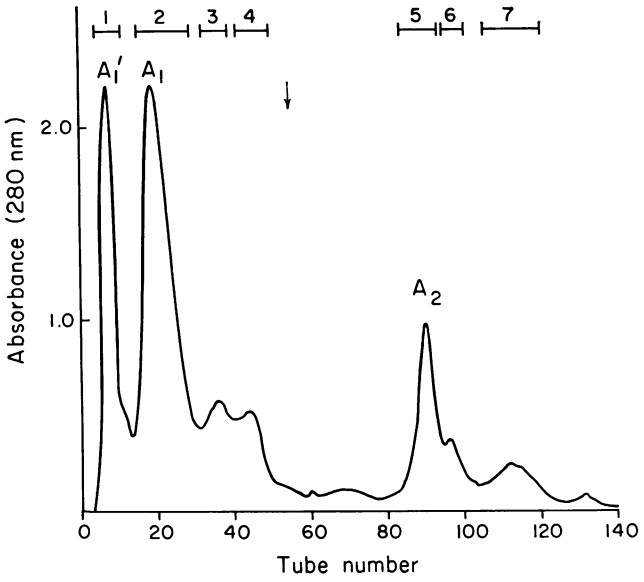

FIG. 3. Ion-exchange chromatography of fraction *2* (Fig. 2*a*) on DEAE-cellulose in 0.02 M Tris (pH 8.0)–8 M urea. At the point indicated by the *vertical arrow*, a linear gradient from 0 to 0.5 M KCl was initiated. Each tube contained 3.0 ml of effluent. The solid line designates the absorbance of effluent fractions at 280 nm.

tryptic digests of the material obtained from the two fractions are shown in Fig. 2*b*. The tryptic digest of material from fraction *2* contains two major peptide components that are absent in the digest of material from fraction *1*, and lacks a third major peptide component that is present in fraction *1*. The maps were otherwise identical. This suggests that the material in fraction *2* represents lower molecular weight fragments of the intact polypeptide chain present in fraction *1*. The molecular weight of the polypeptide chain of Con A must then be about 27,000.

Assuming that the smaller components were the result of degradation of the intact molecule, we attempted to isolate the intact subunit (MW 27,000) by variations in the isolation conditions used, such as buffer composition, temperature, and time of extraction. None of these changed the heterogeneity of the resulting protein sample. Similarly, isolation of Con A directly from whole jack beans gave the same mixture of intact subunit and the fragments. The intact subunit was isolated from the mixture, however, by ion-exchange chromatography on DEAE-cellulose. This material, obtained in 20% yield, is homogeneous on gel electrophoresis in the presence of SDS as shown in Fig. 1. Sedimentation studies in 0.1 M Tris–6 M guanidine·HCl (pH 7.0) gave a molecular weight of 25,800, in agreement with the value estimated from gel electrophoresis experiments. The intact subunit isolated by this method appears to have all the properties of Con A as have been described (3, 10, 11). It is readily crystallizable and it adsorbs to a column of Sephadex G-50 in 1 M NaCl. Assays of its mitogenic activity and hemagglutinating activity showed little difference between this intact subunit and the mixture from which it was isolated.

Fig. 3 shows the results of further fractionation of the material in fraction *2* (Fig. 2*a*) by ion-exchange chromatography in 8 M urea. Approximately equal amounts of material were obtained from fractions *1*, *2*, and *5;* each gave a single component on gel electrophoresis in SDS. Estimation of the molecular weights by sedimentation equilibrium (Table 1) gave values of about 13,000 for A_1, 11,000 for A_1', and 11,000 for A_2, in agreement with their electrophoretic mobilities on the gels.

TABLE 1. *Molecular weight* and amino acid composition† of the intact subunit and fragments A_1, A_1', and A_2*

Amino acid	Intact subunit	A_1	A_1'	A_2
Lys	14.0	10.3	10.1	2.7
His	6.4	2.5	2.3	3.2
Arg	6.4	3.7	3.2	2.7
Asp	36.6	20.3	14.6	12.0
Thr	19.6	12.2	8.5	8.1
Ser	32.1	16.9	15.9	13.8
Glu	13.3	4.9	3.5	7.0
Pro	12.4	6.1	5.0	5.3
Gly	16.4	7.8	7.0	9.4
Ala	20.0	10.6	8.1	10.5
Val	17.4	13.2	11.5	4.7
Met	1.7‡	0.5	0.5	0.6
Ile	15.0	7.9	6.0	5.8
Leu	20.4	9.5	8.5	8.4
Tyr	7.2	2.8	2.3	0.6
Phe	12.7	0.5	0.5	7.4
Total Residues	251.8	129.7	107.5	102.2
Molecular Weight	25,800	12,800	10,600	10,900

* Molecular weights were obtained by sedimentation equilibrium in 6 M guanidine·HCl, pH 7.0.

† Values reported are amino acid residues.

‡ Average of duplicate analyses from 20-hr hydrolysates of performic acid-oxidized samples.

As indicated by their amino acid compositions (Table 1), fragments A_1 and A_1' are similar; however, both differ considerably from fragment A_2, which has a predominance of phenylalanyl residues. This difference was confirmed by two-dimensional electrophoretic peptide maps (13) of the three fragments: the maps of A_1 and A_1' are similar, but they differ considerably from that of A_2. These results suggest that A_1' is a subfragment of fragment A_1. Moreover, comparisons of peptide maps (13) of the intact subunit and a mixture of fragments A_1 and A_2 showed that these two fragments can account for almost the entire molecule. This is also evident from a comparison of the sum of their amino acid compositions with that of the intact subunit. They differ by about 20 residues.

The amino acid sequences of the NH_2-terminus of the intact subunit and fragment A_1 were shown to be identical: Ala-Asx-Thr-Ile-Val. This places A_1 at the NH_2-terminus of the intact polypeptide chain (Fig. 4). Digestion of the intact subunit and fragment A_2 with carboxypeptidase A released the same amino acids, Asn and Ala. Furthermore, digestion of fragment A_2 with trypsin gave a peptide of composition (Asx_2, Pro_1, Gly_1, Ala_1, Leu_3, Phe_1). A peptide of this composition has been isolated from the COOH-terminal cyanogen bromide fragment of Con A (manuscript in preparation) and placed tentatively at the COOH-terminus of the polypeptide chain. Thus, A_2 must span about the last 100 residues of the intact subunit (Fig. 4). These assignments are supported by the results obtained from cyanogen bromide cleavage of the intact subunit

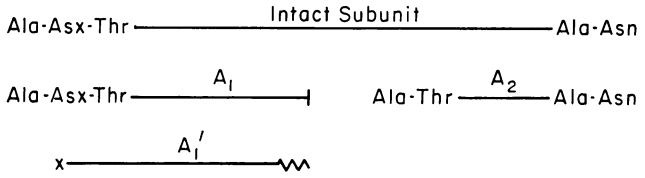

FIG. 4. Arrangement of fragments A_1, A_1', and A_2 with respect to the intact subunit of concanavalin A.

and the isolated fragments, A_1 and A_2 (manuscript in preparation).

DISCUSSION

At present, the mechanism whereby Con A produces its diverse effects on animal cells is obscure. The cell-binding properties of Con A are mainly due to its carbohydrate-binding site. However, the role of Con A in agglutinating cells is unclear because the protein itself aggregates and the cells may be agglutinated by the stacking properties of bound Con A. The mechanism by which Con A acts as a mitogenic agent after it binds to the cell surface is also not known. Burger and Noonan (9) concluded that trypsin converted Con A to a monovalent molecule that covered Con A-binding sites on transformed fibroblasts without agglutinating the cells, thereby converting their growth pattern to that of normal cells. Any mechanism for the action of Con A may be more complicated than previously supposed, however, for we have isolated fragments of the intact subunit of Con A in appreciable quantities from all of the Con A preparations that we have examined. Thus, a single activity may be a property of the intact subunit, the fragments, or some combination of the intact subunit and the fragments. Obviously this finding also has important implications for the determination of the primary and three-dimensional structure of Con A. For these reasons we have constructed a working model of the Con A molecule.

Fig. 5 depicts our schematic model for the possible subunit structures of Con A: *A* is a dimer of intact subunits, each of molecular weight 27,000; *B* is a tetramer constructed from two pairs of subunits; *C* consists of one intact subunit to which the fragments are bound; and *D* is a dimer constructed from two fragmented subunits. Each dimer (*A*, *C*, *D*) has a molecular weight of about 54,000 and a pair of binding sites for manganese ions, calcium ions, and α-methyl-D-glucopyranoside. This model appears to account for all of the known structural features of Con A. We have based our model on the following three key observations.

First, gel electrophoresis in SDS (Fig. 1) and gel filtration in a strongly dissociating solvent (Fig. 2*a*) suggest that Con A isolated by the methods previously used (3, 10, 11) contains several different molecular species. We have isolated from this mixture a polypeptide chain that has a molecular weight of 27,000 by equilibrium sedimentation in guanidine·HCl. Previous data on the molecular weight of Con A in nondissociating solvents gave values of about 55,000 at pH 5.0 (25, 26) and of about 100,000 at higher pH (25, 27). Olson and Liener have estimated the minimum subunit molecular weight of Con A in 8 M urea to be about 16,500 at pH 7.0 (28). From our data, we conclude that the subunit molecular weight of Con A is 27,000. This corresponds to the equivalent weight of Con A in binding manganese ions, calcium ions, and α-methyl-D-glucopyranoside (29), as well as to the crystallographic asymmetric unit (30). At pH values lower than 5.8, Con A exists in solution as a dimer of molecular weight 55,000 (model A). This dimer has three pairs of binding sites: one for carbohydrate and two for metal ions (29). At neutral pH, higher aggregates (e.g., model *B*) of the basic subunit (MW 27,000) are formed.

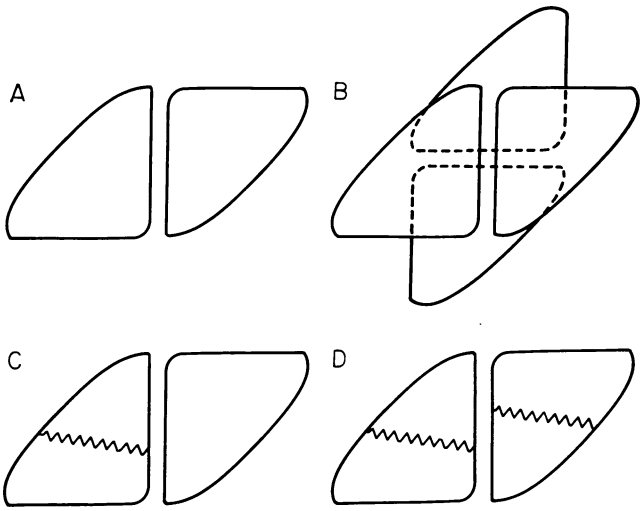

FIG. 5. Schematic model of the structure of native concanavalin A. *A*, a dimer of the intact subunits; *B*, a tetramer of two pairs of subunits; *C*, a dimer of one intact subunit and one fragmented subunit; *D*, a dimer of two fragmented subunits.

In addition, recent crystallographic evidence has shown that the molecule exists as a dimer located on a two-fold axis (30). The pairing of two subunits, each of molecular weight 27,000, on a two-fold axis in our model is consistent with this data, although the possibility of higher molecular symmetry cannot be excluded.

Finally, we have also isolated and characterized three small fragments, A_1, A_1', and A_2, that are present in all preparations of Con A we have examined. Comparative peptide maps of the tryptic digests of the intact subunit and these lower molecular weight components suggest that the smaller components represent fragments of the intact subunit. Further support for this hypothesis is provided by analysis of amino- and carboxyl-terminal residues, which also suggests the positioning of the fragments in the linear structure (Fig. 4). Evidently, the interactions between the intact subunit and the fragments, or among the fragments themselves, are relatively strong. Neither prolonged dialysis nor gel filtration in nondissociating solvents removes the fragments, but they are easily separated from the intact subunit by gel filtration in strongly dissociating solvents. We have, in fact, succeeded in separating this mixture only in 6 M guanidine·HCl or 8 M urea. Thus, we have included in our schematic model pairings of two intact subunits (*A*), one intact subunit to which the smaller fragments are bound (*C*), and a dimer in which the monomeric units are made up entirely of smaller fragments (*D*). We are not yet able to demonstrate whether the fragments have carbohydrate-binding or cell-binding activities, so that the last possibility (*D*) (which seems least likely) cannot be dismissed. Obviously, the tetramer (*B*) might also be constructed from dimers of the type *C* and *D*. The observation that A_1' appears to be a subfragment of A_1, and was obtained in significant yield, suggests that other fragments may also be

components of the Con A molecule as normally isolated. The fact that A_1 and A_2 do not account for the entire molecule supports this conclusion.

The properties of the intact subunit that we have isolated are similar to the original mixture of intact chain and its fragments, suggesting that the intact subunit can indeed exist as the dimer shown in Fig. 5*A*. The fact that this intact chain can be isolated from the mixture in nondissociating solvents lends further support to this hypothesis.

As yet, we have not established the origin of the fragments A_1, A_1', and A_2. They may result from proteolytic cleavage prior to (31) or during the isolation. The possibility that these fragments are normal or abortive gene products is made unlikely by the fact that A_1' appears to be a subfragment of A_1. The presence of unusual linkages in Con A (e.g., esters) that are cleaved either chemically or enzymatically cannot be excluded.

We thank Dr. Hollie N. Pflumm for her advice and help with these experiments and Mrs. Martha O'Connor and Miss Katherine Bergmann for excellent technical assistance.

This work was supported by United States Public Health Service grants AI-09999, AI-09921, and AI-09273 and a Teacher-Scholar Grant from the Camille and Henry Dreyfus Foundation.

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