Indoleacetic Acid Synthesis by Polyphenols in the Extraction of Pinus Phloem and Cambial Tissue

Abstract. *Indoleacetic acid may be formed by polyphenol oxidation of tryptophan during extraction of plant tissue with aqueous or organic solvents. Polyphenoloxidase promotes the conversion, but studies with heat-inactivated tissue indicate that the reaction can proceed without enzymes.*

Indoleacetic acid can be produced from naturally occurring tryptophan and polyphenols in plant tissue when they are mixed by cell disruption during extraction. It is probable that the auxin is produced as an artifact in ether extracts of *Pinus* phloem and cambial material.

Gordon and Paleg (*1*) showed that indoleacetic acid can be formed in vitro from tryptophan and catechol or other *o*-dihydroxy phenols. Tryptophan is oxidized by the polyphenols to indolepyruvic acid, which undergoes spontaneous oxidation to indoleacetic acid. The conversion is catalyzed by polyphenoloxidase, but can also proceed without the enzyme, provided that the *p*H of the system is high enough to allow sufficient autoxidation of the polyphenols.

Three experiments were designed to examine the possibility of indoleacetic acid synthesis during extraction. Phloem and cambial tissues of *Pinus sylvestris*, *P. resinosa*, and *P. taeda* were used.

The yield of indoleacetic acid extracted in the presence of ascorbic acid was compared with the yield obtained at basic *p*H. Ascorbic acid inhibits the formation of quinones which are supposed to complete the oxidation of tryptophan, while basic conditions promote their formation (*1*). Thus, lower yields of indoleacetic acid in the presence of ascorbic acid than in an alkaline-buffered system may be explained by polyphenol oxidation of naturally occurring tryptophan.

Tissue was removed from fresh stem bolts, weighed, and placed into either 0.2*M* ascorbic acid or tris buffer at *p*H 9.0. The suspended material was homogenized for 2 minutes in a blender and strained through cheesecloth. The liquid so obtained was centrifuged to remove fragments of the cell walls. The acid fraction of the supernatant was recovered by bicarbonate partitioning (*2*), and placed on a paper strip for chromatographic partitioning with a mixture of isopropanol, ammonia, and water as the solvent. Determination of the indoleacetic acid fractions were made colorimetrically (*1*). The mean yield of indoleacetic acid in three paired extractions was 0.13 μg per gram of fresh tissue in the presence of ascorbic acid. In the high *p*H treatment, the mean yield was 0.26 μg of indoleacetic acid per gram of fresh tissue. In addition to its action as a reducing agent, ascorbic acid inhibits autoxidation of polyphenols by its buffering action at about *p*H 2.8.

Extracts from heat-inactivated tissue were compared with those of untreated tissue to test the effect of polyphenoloxidase on the synthesis of indoleacetic acid. To inactivate enzymes, stem bolts were submerged in boiling water for 5 minutes before excision of the tissue. Paired tissue samples from heat-treated and untreated bolts were extracted in ice cold ether in a blender for 2 minutes. The extract was filtered and partitioned with bicarbonate (*2*) to recover the acid fraction. After further partitioning with paper chromatography, the indoleacetic acid was estimated colorimetrically (*1*).

The mean yield of two heat-treated samples was 0.09 μg of indoleacetic acid per gram of fresh tissue; the yield of untreated tissue was 0.21 μg per gram of fresh tissue. The lowered yield with heat treatment was similar to the lowered yield with ascorbic acid. Apparently, conditions affecting the enzyme or affecting the oxidation and reduction of polyphenols affect the yield of indoleacetic acid.

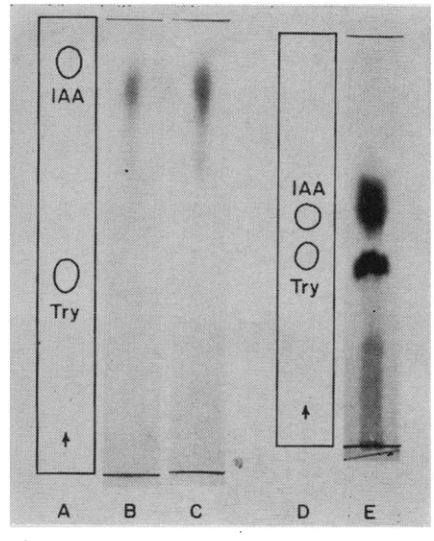

Fig. 1. *A*, Paper chromatogram of nonradioactive indoleacetic acid and tryptophan developed in isopropanol, acetic acid, and water. *B*, Autoradiogram of the acid fraction of an ether extract of untreated tissue to which C^{14}-tryptophan has been added. Same solvent as in *A*. *C*, Same as *B*, except that the tissue was heat treated. *D*, Chromatogram of nonradioactive indoleacetic acid and tryptophan, developed in isopropanol, ammonia, and water. *E*, Autoradiogram of the whole ether extract of untreated tissue, C^{14}-tryptophan added.

The synthetic system was further tested by adding C^{14}-tryptophan to both fresh and heat-treated tissue before extraction. Plugs 1.3 cm in diameter were removed from the main stem of a standing *P. sylvestris* tree; phloem and cambial derivatives were excised. Half of the individual samples were heated in boiling water for 5 minutes. Each sample was ground in a mortar with 2 ml of ether, 1 μc of C^{14}-tryptophan in 1 ml of 80 percent ethanol, and a little sand. The macerated tissue suspension was kept in a freezer for 2 hours. The ether was filtered off and partitioned with bicarbonate (*2*). A paper chromatogram of the acid fraction was developed in a mixture of isopropanol, acetic acid, and water (4 : 1 : 1), a solvent known to give a wide separation between tryptophan and indoleacetic acid. Strips were autoradiographed after development (Fig. 1, left).

In a similar test, a small sample of fresh phloem material was extracted with ether in the presence of 1.5 μc of C^{14}-tryptophan. In this case, the ether extract was not partitioned, but the whole extract was condensed, chromatographed, and autoradiographed (Fig. 1, right).

Bioassays of the strips used for autoradiography were not made; however, the zones containing spots identified as indoleacetic acid have the same rate of movement on the paper as those exhibiting growth activity in other tests of pine phloem extracts. They also correspond to the rate of movement of authentic indoleacetic acid run concurrently and tested with standard indole reagents. Regardless of whether or not the zones assumed to be indoleacetic acid are indeed that, the chromatograms show that free tryptophan is altered by the tissue constituents.

The results of the three experiments taken together strongly suggest the operation of the polyphenol system in these *Pinus* extracts. We believe that this conversion may explain the lack of agreement between auxin estimates based on diffusion and on extraction of similar tissues, as reported by Steeves

and Briggs (*3*). Wightman (*4*) and Dannenburg and Liverman (*5*) have reported the conversion of tryptophan to indoleacetic acid by plant tissue. Both studies entailed the uptake of C^{14}-tryptophan and extraction of ground or homogenized tissue, and the authors assume a normal enzymic conversion by an uncharacterized system for synthesizing indoleacetic acid. However, unless *o*-dihydroxy phenols are rigorously excluded from tissues, there is the possibility that these are responsible for the conversion.

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References and Notes

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Gamma Globulin: Unmasking of Hidden Antigenic Sites on Light Chains

Abstract. *Normal light (L) chains cleaved from normal pooled human gamma globulin contain immunologic determinants not overtly present in the native unreduced molecule. These hidden determinants are shared with Bence Jones proteins. Similarly, one of two Bence Jones proteins contained immunologic determinants which were not overtly present on the autologous serum myeloma protein.*

Studies of Bence Jones (BJ) proteins have shed some light on the basic immunological, structural, and genetic nature of the human immunoglobulins. The two major antigenic groups of Bence Jones proteins have immunologic counterparts in the normal populations of γ_2-, γ_{1A}-, and γ_{1M}-globulins (*1*). In addition, Bence Jones proteins appear to be structurally identical to light (L) polypeptide chains cleaved from the autologous pathologic serum protein (*2, 3*). Using group specific antibodies to Bence Jones protein, we have previously demonstrated individual specificity of the immunizing proteins (*4*). We have also shown that Bence Jones proteins of the same group from different patients contain certain antigenic determinants not overtly present in pooled human 7*S* γ-globulin from normal individuals. The immunologic difference between Bence Jones protein and normal γ-globulin might be due not only to a quantitative change, but also to a qualitative abnormality resulting from any of the following, either individually or in combination, (i) preparative artifact; (ii) unmasking of hidden antigenic sites; or (iii) primary structural difference that might be expected of an abnormal protein. The following studies were undertaken to investigate the possibility that Bence Jones proteins contain antigenic determinants present in normal L-chains, but inaccessible when the L-chain is incorporated into the γ-globulin molecule.

Bence Jones proteins were typed (*5*) and isolated from urine by precipitation with ammonium sulfate; they were purified by dialysis, lyophilization (*6*), and finally by Pevikon block electrophoresis (*7*). Specific antibodies to group 1 and group 2 Bence Jones proteins were prepared in rabbits (*4*). Serums containing myeloma proteins were used without further separation of the protein. Immunologic analyses were performed by double diffusion in agar.

Normal L-chains (*8*), S (slow) fragments (*8*), pooled human γ-globulin (*9*), and two group 1 myeloma serum proteins were compared by means of specific antibody to group 1 Bence Jones protein (anti-BJ 1–40). The S (slow) fragments were the electrophoretically slow components of a papain digest of normal γ-globulin. One myeloma protein was a γ_2- and the other, a γ_{1A}-type. The specific antibody was absorbed with 5 mg of lyophilized normal serum to remove reactivity that arises from contamination of the immunizing antigen by slight amounts of normal serum protein. Reactivity was of significant degree with the normal L-chains and the two myeloma proteins, only slight with the normal γ-globulin, and absent with the S fragment (Fig. 1). Similar findings were noted when L-chains, γ-globulin, S fragments and group 2 myeloma proteins of γ_2-type were compared by testing with a specific antibody to group 2 Bence Jones protein. These studies indicate that immunologic information present on normal L-chains may be hidden or inaccessible in the S fragments of papain-split γ-globulin or in the undigested γ-globulin molecule.

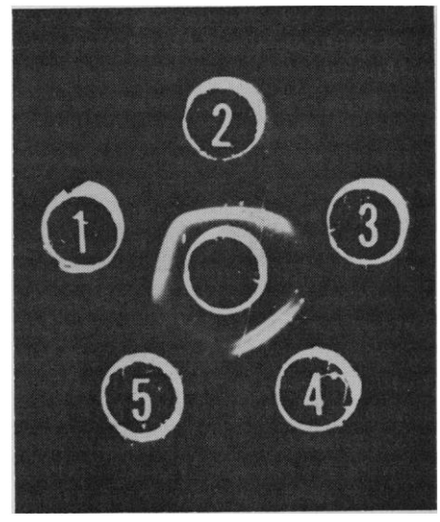

Fig. 1. Immunodiffusion: Comparison of L-chains, S fragments, γ-globulin, and myeloma proteins. Well No. 1, γ_{1A}-myeloma protein 1–40; No. 2, γ_2-myeloma protein 1–41; No. 3, normal S fragments; No. 4, normal L-chains; No. 5, normal pooled human γ-globulin. Center well contained a specific group 1 Bence Jones (1–40) antibody absorbed with 5 mg of lyophilized normal human serum per milliliter. Protein concentration in the antigen wells was 0.1 percent.

In view of these findings two Bence Jones proteins were compared in an analogous sense to the autologous γ_2 myeloma proteins by reactivity with specific antibody to group 2 (anti-BJ 2–20). In this study (Fig. 2) the antibody was absorbed with normal serum and also with 2 mg of pooled normal human γ-globulin per milliliter. The immunizing Bence Jones protein (BJ 2–20) and its autologous myeloma serum protein showed an identical degree of reactivity. Cross-reactivity of the specific antibody was noted with a Bence Jones protein (BJ 2–24) from another patient; but no reaction was apparent with the myeloma protein from this patient. These findings suggest that BJ 2–24, BJ 2–20 and myeloma protein 2–20 share antigenic determinants which are not overtly present on the myeloma protein 2–24. These determinants shared by Bence Jones proteins of the same group but from different patients represent a common core of antigenic determinants characteristic of the major immunologic group (*4*). Bence Jones proteins and myeloma proteins from the same patient should share the same antigens. The discrep-