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Conference paper

FRACTAL DIMENSION OF LIGNIN STRUCTURE AT THE MOLECULAR LEVEL

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Mechanisms of synthesis and structural organization of lignin, a structural polymer of the plant cell walls, have not been completely elucidated. In this study we applied fractal analysis to the images of lignin polymer obtained using scanning tunneling microscope. The analysis showed the regularity of the polymer at different levels of organization. We obtained fractal dimension 1.95±0.02 for the lignin polymer. There is no significant

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difference, at the confidence level 95%, in the fractal dimension between images representing different organizational levels of lignin. That shows that lignin produced in *in vitro* condition has a fractal organization and, consequently, one could expect a similar regularity of the polymer in *in vivo* conditions. The value of the fractal dimension is in good agreement with the theoretically predicted value.

Key words: cell walls, fractal dimension, free radical polymerization, phenolic alcohols, scanning tunneling microscopy, self-similarity

Lignin, a structural polymer of the plant cell walls and the second abundant natural polymer on Earth, is formed through free radical polymerization of phenolic alcohols (coniferyl, *p*-coumaryl and synapyl alcohol) catalyzed by different peroxidases. It is widely accepted that lignin has a protective role in the plant cell towards different kinds of stress (Lewis and Yamamoto, 1990). However, much remains unknown about the mechanism of synthesis and structural organization of lignin, and especially about regularity of the polymer structure in the cell wall.

The principal concept of fractal geometry is based on the fact that most shapes in nature cannot be described well enough using ideal constructions of Euclidean geometry, because they are relatively "irregular". Since an important characteristic of fractal geometry is the property of "self-similarity", in the statistical sense, fractal structure should have a constant fractal dimension as the image is viewed at different levels of magnification, i.e. different range of scale (Gluck, 1986; Falconer, 1990). These facts were used for development of methods for "complexity measuring" of different natural shapes (Cross *et al.*, 1994; Glenny *et al.*, 1991). In this paper, we used the images of an enzymatic lignin polymer, obtained using a scanning tunneling microscope (STM), for determination of polymer fractal dimension. Since it is known that fractal objects or clasters are formed in the conditions of diffusion-limited particle aggregation (Smirnov, 1991), and diffusion has an important role in lignin biosynthesis, the fractal theory can prove useful in obtaining new data about polymerization laws of lignin precursors. On the other hand, fractal analysis of a lignin polymer is a contribution to the understanding of its structural organization.

Reaction mixture for enzymatic lignin synthesis contained $5 \cdot 10^{-3}$ M coniferyl alcohol, $5 \cdot 10^{-3}$ M H_2O_2 and $2.5 \cdot 10^{-8}$ M horseradish peroxidase, in $5 \cdot 10^{-2}$ M phosphate buffer pH 7.6. The polymer structure was studied two days after the start of polymerization. The STM images of polymer were obtained using a commercially available model of the microscope (Nanoscope II, Digital Instruments). The images were recorded using a Pt/Ir tip which was mechanically sharpened. Highly oriented pyrolitic graphite (HOPG) plates were used as substrate for polymer samples (Radotić *et al.*, 1994). The previously published STM images of lignin polymer (Radotić *et al.*, 1994; Radotić *et al.*, 1998) were used for determination of its fractal dimension. The micrographs were scanned and prepared for analysis using Aldus Photo Shop Styler 2.0. The digitized images of polymer were converted to a graphic form that is more suitable for manipulation. Digitalized polymer shapes were analyzed by a box-counting (square-covering) method (Budimlija, 1998; Peitgen *et al.*, 1992), superimposing them on a succession of square grids, containing increasing number of squares (with a decreasing edge length of each of them), in order to test invariability of the shape in different scale range. The number of grid squares (boxes) that contact a polymer image were counted. The fractal dimension was determined according to the formula:

$$D_B = \lim_{\varepsilon \to 0} \frac{\log N(\varepsilon)}{\log 1/\varepsilon}$$

where ε is the side length of one box within the grid, and N(ε) is the smallest number of boxes of side length ε required to completely cover the border of the object being measured (Smith *et al.* 1989). In a log-log plot of the (1/ε) (x-axis) versus N(ε) (y-axis) the linear approximation of the first degree polynomial (y=a+bx) was performed, where the inclination (B) of the least square fitted line presents the box-counting fractal dimension (Fig. 1). The obtained fractal dimensions of lignin at different organization levels were statistically analyzed applying analysis of variance (ANOVA) for a level of reliability of 95% (p<0.05).

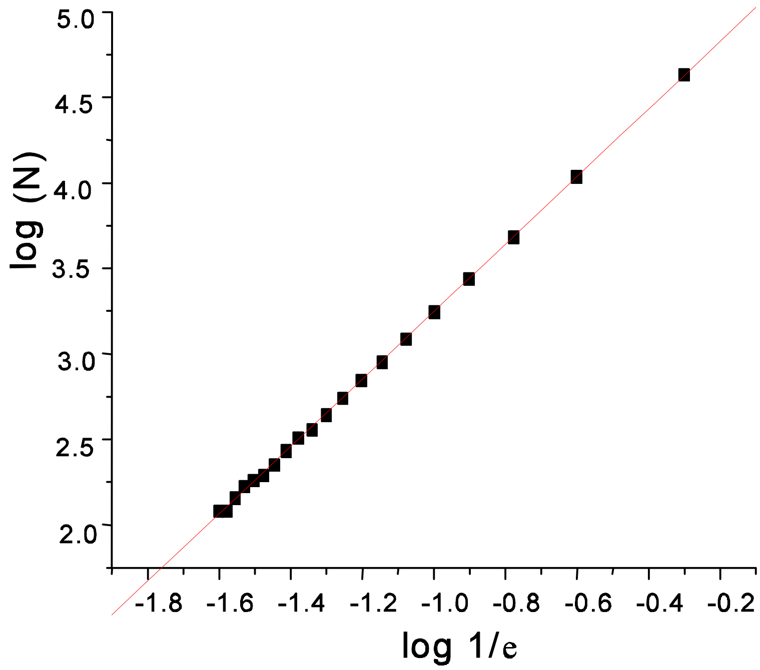

Fig. 1. Log-log plot of the (1/ε) (x-axis) versus N(ε) (y-axis) and linear approximation of the first degree polynoma (y=a+bx); the inclination (b) of the least square fitted line presents the box-counted fractal dimension of the polymer

The mean fractal dimension of the lower organization level of lignin polymer, obtained using the image in Fig. 2, is D = 1.94309±0.019027. The mean fractal dimension of polymer at the higher organization level obtained using the image presented in Radotić *et. al.*, (1994) and Radotić *et. al.* (1998) is D = 1.95645 ± 0.02039 (Fig. 1). The results show that, at the level of 95% (p<0.05), there was no significant difference between the groups of analyzed images at different levels of structural organization. That means that lignin produced *in vitro* has fractal structural organization. This fact gives the new possibility of using the fractal analysis method to study the

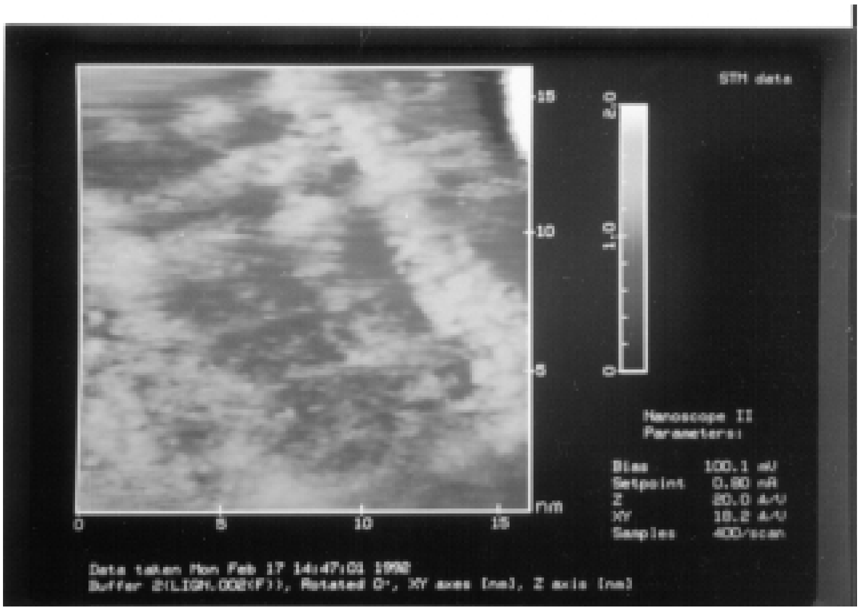

Fig. 2. STM image of the lower organization level of the enzymatic lignin polymer; U=100.1 mV, I_T=0.83 mA

differences between various polymerization mechanisms and structural differences of corresponding lignin polymers. Fractal structural organization of lignin confirms high regularity within the polymer, as seen at the STM images (Radotić *et al*., 1994), and also shows that regularities at different structural levels are interrelated. This result confirms that the lignification even in *in vitro* conditions is highly ordered. A group of authors has recently shown the fractal structure of lignin obtained *in vitro*, using indirect, hydrodynamic methods (Karmanov and Monakov, 1995). They obtained two different values of fractal dimension, depending on the mechanism of polymer synthesis. In this work we have shown for the first time directly the fractal organization of lignin on the basis of its images. The obtained fractal dimension of lignin differs from fractal dimension of mentioned authors, reflecting the difference in the mechanism of synthesis. However, it is much closer to their finding of D=1.66±0.16 for bulk polymerization than for end-wise polymerization. End-wise polymerization is diffusion-limited association of modules in which radicals join the growing macro-molecule in subsequent steps. Bulk polymerization or module-module association corresponds to the formation of a great number of reaction centers which are prone to mutual associations. In this case the reaction rate is highest at the beginning of the synthesis. Theoretical calculation for the bulk model (Karmanov and Monakov, 1995) is still closer (D=1.78±0.06) to our calculated value of fractal dimension (D=1.96±0.02). The fractal analysis applied on digitalized polymer shapes is a reliable method that can be used for morphometrical purposes both in routine work and research as well.

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