

## RAPID COMMUNICATION

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## Novel paper strength additive containing cellulose-binding domain of cellulase

Received: March 7, 2001 / Accepted: April 18, 2001

**Key words** Cellulose-binding domain · Cellulase · Anionic polyacrylamide · Paper strength additives · Paper stock conductivity

### Introduction

Paper strength characteristics inherently depend on the single-fiber strength, the fiber-to-fiber bonds, and the fiber network forms such as the sheet formation. The roles of paper strength agents have recently become more significant because of the utilization of pulp wood with poor quality and recycled fibers due to environmental protection and resource recovery. Polymeric additives that are commercially available are natural, partially modified, or synthetic polyelectrolytes, for example, cationic starches, polyacrylamides (PAMs), polydiallyldimethylammonium chloride, and polyamideamine-epichlorohydrin (PAE). These ionic water-soluble polymers have long been used in the practical paper-manufacturing processes, although a variety of substances that accumulate in the white water of the paper mill have interfered in the performance of these polymers. The effective adsorption of additives on the pulp fibers is obstructed by large amounts of inorganic ions and anionic trash in the furnish, such as newsprint paper stock.<sup>1</sup> Nonionic polymer systems using polyethylene oxides and phenol formaldehyde resins have been applied to improve the fines' retention and drainage efficiency under industrial conditions,<sup>1,2</sup> but they make nearly no contribution to paper strength enhancement.

As one of the fundamental solutions to overcome the inevitable difficulties when polyelectrolytes are used as pa-

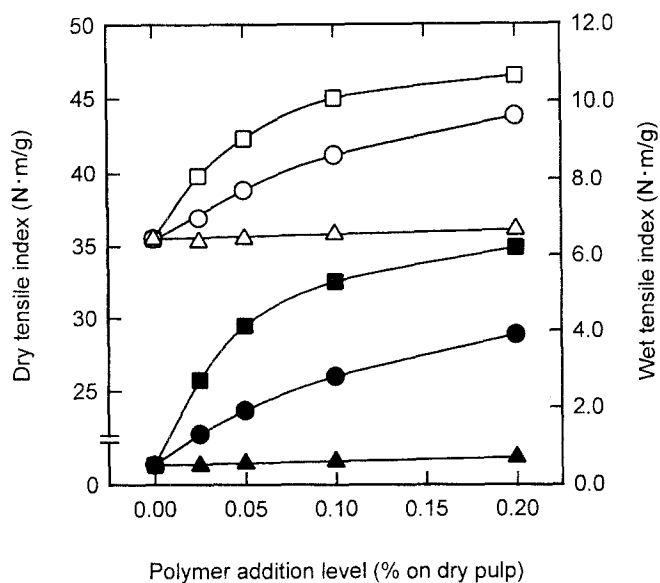
permaking additives in contaminated papermaking systems, we tried to strike a new note by utilizing the function of a cellulose-binding domain (CBD) of cellulase. Cellulases catalytically hydrolyze the cellulose and cellulosic materials in an aqueous medium, and they consist of at least two structural domains: a catalytic domain (CD) and a CBD.<sup>3</sup> Interestingly, it was reported that CDs split by limited proteolysis could not attack insoluble celluloses, although soluble cellulosic substrates were easily hydrolyzed,<sup>3,4</sup> and isolated CBDs were quasi-irreversibly adsorbed on microcrystalline cellulose without showing any catalytic activity.<sup>5</sup>

In this study, CBDs separated from a commercial cellulase using papain were covalently introduced into anionic PAM (A-PAM) with small numbers of carboxyl groups. The A-PAM combined with the CBDs, called CBD-A-PAM here, was subjected to handsheet-making as an internal additive, and tensile strength improvements of the handsheets were investigated with regard to polymer addition levels and paper stock conductivity.

### Materials and methods

The CBDs were obtained from a commercial cellulase containing 1,4- $\beta$ -D-glucan cellobiohydrolase (EC 3.2.1.91) and 1,4- $\beta$ -D-glucan 4-glucanohydrolase (EC 3.2.1.4) produced by the fungus *Trichoderma viride* according to the following methods. Papain 1 mg/ml was activated at 37°C for 30 min in phosphate buffer pH 6.5. Part of the activated papain solution was poured into cellulase solution (30 mg/ml) with a cellulase/papain ratio (w/w) of 30:1; the mixture of enzymes was kept at 37°C and pH 6.5 for 4 h.<sup>3</sup> The cellulase digest was gel-filtrated using Sephadex G-100 (Amersham Pharmacia Biotech, UK), and the CBDs were finally obtained in about 5% (w/w) yield. Separation of the CBDs was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Next, 20 ml of 0.1% (w/w) A-PAM (HH-351; Kurita, Japan) with an average molecular weight of  $4 \times 10^6$  and a charge density of 0.83 mEq/g, which homogeneously contained 10 mM of

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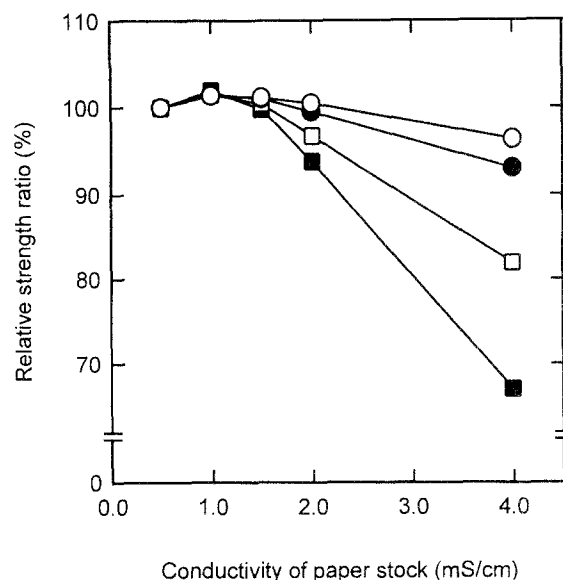


**Fig. 1.** Tensile strength index of handsheets prepared with cellulose-binding domain-anionic polyacrylamide (CBD-A-PAM) (circles), A-PAM (triangles), or polyamideamine-epichlorohydrin (PAE) (squares) (0%–0.2% on dry pulp). Open and filled symbols indicate the dry and wet tensile indices, respectively

1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) hydrochloride, was kept at pH 4.75 for 30 min; the CBDs (ca. 0.1 g) were then gradually added to the A-PAM/EDC solution at room temperature for 4 h.<sup>6</sup> Designated amounts of CBD-A-PAM, A-PAM, or PAE (Japan PMC, Japan) were added (0%–0.2% based on dry weight of pulp) to a 0.15% suspension of bleached hardwood kraft pulp that had been beaten to 450 ml of Canadian Standard Freeness. Conductivity of the pulp suspension was adjusted to about 0.5–4.0 mS/cm by adding a dilute  $\text{CaCl}_2$  solution before the polymer additions. Handsheets with a basis weight of 60 g/m<sup>2</sup> were prepared according to TAPPI test methods.<sup>7</sup> The handsheets after being cured at 105°C for 30 min and then conditioned at 20°C and 65% relative humidity for more than 24 h were subjected to tensile strength tests under dry<sup>8</sup> and wet<sup>9</sup> conditions. All enzymes and chemicals (Wako, Japan) excluding A-PAM and PAE were reagent grade.

## Results and discussion

Figure 1 illustrates the dry and wet tensile strength index of handsheets prepared with CBD-A-PAM, A-PAM, or PAE (0%–0.2% addition) at paper stock conductivity (0.5 mS/cm). The practical PAE resin for improving the wet strength in the paper-manufacturing industry was used as a control of paper strength additive. Increasing the polymer addition levels of both CBD-A-PAM and PAE enhanced the dry tensile strengths of the paper sheets. Wet strength developments also appeared after the internal CBD-A-PAM addition, being inferior to those after the PAE addition. The A-PAM addition did not contribute to the improvement of



**Fig. 2.** Influence of paper stock conductivity on the tensile strength of handsheets prepared with CBD-A-PAM (circles) or PAE (squares) (0.2% on dry pulp). The relative strength ratio corresponds to the percentage calculated by dividing each tensile index by the original one at initial stock conductivity (0.5 mS/cm). Open and filled symbols indicate the dry and wet relative strength ratios, respectively

dry and wet paper strength, as the A-PAM without cationic retention aids could not be adsorbed on pulp fibers by ionic repulsion between the A-PAM and the fibers. Thus the CBD-A-PAM performed well as an internal paper strength agent, and these obvious effects implied that the water-soluble carbodiimide EDC could covalently link primary amines of the CBDs with carboxyl groups of the A-PAM consisting of acrylamide and acrylic acid.

Based on the mechanisms of paper strength improvement with the epoxidized polyamide resins, homo-crosslinking between PAE resins, co-crosslinking of the resins with carboxyl groups of pulp fibers, or both have been proposed.<sup>10</sup> However, no covalent bonds analogous to those seen with the epoxidized polyamide resins occurred between the CBDs and the cellulosic fibers. Therefore, quasiirreversible binding of the CBDs with the fibers must contribute to paper strength enhancement.

Figure 2 demonstrates the impact of paper stock conductivity on the strength properties of paper sheets prepared as a 0.2% addition. In the conductivity range of 0.5–1.5 mS/cm, the tensile strengths of paper sheets prepared with CBD-A-PAM or PAE varied little under the dry and wet testing conditions, though higher conductivity (>2 mS/cm) reduced the PAE performance especially in the wet state. The strength properties of the CBD-A-PAM-added sheets remained almost unchanged compared with those of the PAE-added sheets with higher stock conductivity (up to ca. 4 mS/cm). It was reported that divalent cations such as  $\text{Ca}^{2+}$  in the fiber stock greatly disturbed PAE retention on paper sheets and so decreased the paper strength because the cations adsorbed on the pulp weakened the anionic charges of the fibers.<sup>11</sup> In contrast, the interactions between CBD-A-PAM and the pulp fibers were presumed to be similar to

those in enzyme–substrate complexes, and thus the polymer–pulp complexes would be stabilized by the binding power of enzymes toward substrates even in an aqueous system with a high salt concentration. These results strongly suggest that the adsorption systems dependent only on ionic interactions between wet-end additives and pulp fibers become ineffective under contaminated conditions in recent papermaking circumstances. On the other hand, CBD polymers can be expected to be of great advantage for practical application because the adsorptivity of the CBD polymers onto pulp fibers results from the plural synergistic interactions such as ionic attraction, hydrogen bond, hydrophobic bond, and van der Waals force, which cooperate each other.<sup>12</sup> Moreover, the CBD polymers may have a strong affinity for pulp fibers, and colloidal disturbances that frequently spoil the effects of the internal polymer additions may not interfere with the effectiveness of the CBD polymers.

Strength characteristics of CBD-A-PAM-added sheets have not been satisfied, and synthetic methods to prepare CBD polymers have not been established. Nevertheless, novel CBD polymers used as papermaking additives, whose development will be advanced through molecular design by mimicking the unique function of CBDs, are considered promising materials for contaminated papermaking processes in the near future.

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