

The Corn Inhibitor of Activated Hageman Factor: Purification and Properties of Two Recombinant Forms of the Protein*1

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A cDNA clone that encodes the 14-kDa bifunctional inhibitor from corn seeds (L. Wenet al., *Plant Mol. Biol.* 18, 813–814, 1992) has been expressed in *Escherichia coli* after being incorporated into the pT7 expression vector. This inhibitor protein, referred to as CHFI (for the corn inhibitor of activated Hageman factor) or as the popcorn inhibitor, is an important tool for specific inhibition of human activated Hageman factor (activated forms of coagulation Factor XII) and has been well characterized as isolated from corn seeds. Recombinant CHFI was expressed in *E. coli* in high levels but was insoluble. We solubilized the expressed protein by sonication in 5 M urea and 1% Triton X-100. Several steps of purification, culminating with reversed-phase HPLC, yielded pure, recombinant corn inhibitor in about 5% yield (about 1 mg per liter of culture). The form with which we have worked most, 7N-CHFI, contains 7 amino acid residues at its N-terminus that are encoded by the expression vector. Physical properties of this recombinant protein indicate it has the expected mass and is properly folded. Functionally, 7N-CHFI is indistinguishable from the inhibitor isolated from corn seeds in its inhibition of porcine trypsin, human β -Factor XIIa, failure to inhibit human plasma kallikrein, and its inhibition of an insect α -amylase. A second recombinant form, (4N-11)-CHFI, which lacks 11 residues from the corn inhibitor's N-terminus, is indistinguishable from 7N-CHFI in its pattern of inhibition of the three test proteinases but is inactive against the insect α -amylase. This suggests that the N-terminal region of 7N-CHFI forms at least part of the protein's site of interaction with α -amylase.