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High-throughput analysis of the cereal protein functions by improvement of Cleveland peptide mapping

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Objectives

We report a simple and relatively rapid sample preparation method for Cleveland peptide mapping and its applications identify the seed embryo and lamma proteins separated by 2-DE in cereal proteome analysis.

Materials and Methods

1) Materials: Rice embryo and wheat lemma
2) Cleveland peptide mapping: The gel pieces were chopped into smaller pieces and inserted in the sample well of the stacking gel for SDS-PAGE. One hundred ml of the electrode solution was added to the dried gel pieces. After incubation for 1 h, 20 ml of 2X diluted SDS sample buffer containing 10 ml of *Staphylococcus aureus* V8 protease (Pierce, Rockford) (0.1 mg/ml) in deionized water was overlaid on the sample solution. Electrophoresis was then continued and the separated digests were electroblotted on the PVDF membrane and subjected to gas-phase sequencing.

Results and Discussion

Using this method, the peptide mapping of the three standard proteins, bovine alpha-lactalbumin, soybean Kunitz trypsin inhibitor, and bovine carbonic anhydrase, was also performed. We applied 100 pmol of the proteins to the SDS-PAGE gels and recovered 7.3-13.5 pmol as initial yields of the peptides in gas-phase sequencing. This indicates that, since the amino acid sequence of 1 pmol of protein can be determined by the gas-phase sequence if at least 14 pmol of protein is available, then we can determine the sequence. This method is not only sensitive, but also efficient. We are able to perform peptide mapping of 20 proteins per day. In the present study, this method was applied to identify a number of the seed embryo proteins in the rice and the lemma proteins in the wheat proteome analysis.

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