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Primary Structure of Rat Spermidine Synthase: An Example of Refining the cDNA-Derived Amino Acid Sequence

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The primary structure of rat spermidine synthase having the N-terminal acetylated methionine and 98.7% homology with that of the mouse enzyme is presented using a limited amount of the homogeneous enzyme. The study strategy was principally to compare the molecular masses of liberated peptides determined by three specific cleavage methods with those expected from known cDNA-derived amino acid sequences of mouse and human enzymes using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF-MS). The cleavage methods involved two enzymatic methods using lysylendopeptidase and arginylendopeptidase, and a chemical method for cleaving at the cysteine residue using 2-nitro-5-thiocyanobenzoic acid. Their usefulness was clearly demonstrated. Column-switching semimicro reversed-phase HPLC, which permits application of the entire reaction mixture, was useful for collecting a small amount of peptides containing the N-terminal amino acid, to confirm acetylation of the N-terminal methionine by MALDI TOF-MS. It was necessary in this approach to examine the amino acid sequence of certain peptides. The Edman method was used for the sequence analysis, and this will be replaced by an improved MALDI TOF-MS now available in a few laboratories.