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Preparation and Usefulness of Some Fluorogenic Substrates for Assay of Arginyl-tRNA-Protein Transferase by HPLC

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A series of fluorescent substrates and products was prepared and evaluated for the assay of arginyl-tRNA-protein transferase (arginyltransferase) activity by HPLC. Since N-aspartyl-N'-dansylamino-1,4-butanediamine (Asp(4)DNS) was the most suitable substrate of the compounds tested, which had a three-, four-, or five-methylene-chain interval between Asp or Glu and DNS, the following enzymatic studies were focussed on Asp(4)DNS and its product, N-arginylaspartyL-N'-dansylamino-1, 4-butanediamine(ArgAsp(4)DNS). The apparent Km value for Asp(4)DNS was calculated to be 30 μM using a hydroxyapatite-treated arginyltransferase preparation from hog kidney, which was free from any enzyme that might decompose the two compounds. The present HPLC method was shown to be advantageous in reliability and sensitivity compared to the available isotope paper disk method using the hydroxyapatite-treated enzyme preparation and in applicability to crude samples examined using a DEAE-treated arginyltransferase preparation and 105,000g supernatant (105S) from hog kidney. Stepwise elimination of Arg and Asp from ArgAsp(4)DNS was observed with the two crude enzyme solutions, and the elimination of Arg was suppressed by the addition of bestatin, suggesting the participation of certain aminopeptidases. Although Asp(4)DNS was decomposed significantly with 105S, an incubation-time-dependent linear elevation of ArgAsp(4)DNS was maintained for 5 min in the presence of bestatin. Furthermore, an addition-recovery test of the DEAE-treated enzyme preparation for the 105S assured accurate determination of arginyltransferase activity in the 105S under the tentatively established conditions. The present HPLC method, which

permits the simultaneous determination of 4-dansylamidobutylamine, Asp(4)DNS, and ArgAsp(4)DNS, was advantageous in measuring arginyltransferase activity and detecting the presence of unfavorable enzyme(s) in samples to ensure accurate determination.