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FIBER-ENTRAPPED DIPEPTIDYL AMINOPEPTIDASE

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The enzyme dipeptidyl aminopeptidase has been immobilized on cellulose triacetate fibers. The Michaelis–Menten parameters of the entrapped enzyme have been determined and compared to those of the literature for the native enzyme. The entrapped enzyme retains 13% of its activity toward synthetic substrate. The influence of mixing effects on the catalytic properties of the enzyme has been substantiated. The activity appears to be controlled by external mass-transfer.

INTRODUCTION

Dipeptidyl aminopeptidase (Cathepsin C) (1–3) is known to catalyze the consecutive removal of dipeptide moieties from the unsubstituted amino termini of polypeptide chains (4). Promising enzymatic methods for sequential analysis of polypeptides have recently made use of this enzyme (5).

On the other hand, the advantages of having enzymes immobilized by trapping into insoluble matrices are well documented. Particularly encouraging in this respect is the possibility of trapping enzymes into triacetate cellulose fibers (6).

In this communication we report on the entrapment of dipeptidyl aminopeptidase (DAP I) in cellulose triacetate fibers. The enzymatic activity of the immobilized enzyme is compared to the activity of the native enzyme.

MATERIALS AND METHODS

Bovine spleen DAP I was purchased from Sigma Chemical Company, St. Louis, Missouri. For entrapment, 16 mg of the commercial lyophilized powder, containing 8 mg of DAP I, was dissolved in 4 ml of water–glycerol (70 : 30, vol/vol). The protein solution was emulsified with cellulose triacetate dissolved in methylene chloride (7). The emulsion was extruded through a spinneret into a bath containing toluene, where the polymer coagulated as a filament which entraps the microscopic droplets of aqueous protein solution. The fibers were air-dried and stored at 4°C.

Cysteamine hydrochloride and Gly-L-Phe-β-naphthylamide (Gly-Phe-NA) were purchased from Serva Feinbiochemica, Heidelberg, Germany. The assay for enzyme activity against Gly-Phe-Na was carried out using a fluorometric method (4). The following stock solutions were prepared in 50 mM sodium citrate buffer, pH 6.0: (1) 0.25 mM Gly-Phe-NA; (2) 60 mM β-mercaptoethylamine; (3) 1 μmol/ml β-naphthylamine (NA) (standard solution); (4) 0.1 mg/ml DAP I in water or in the citrate buffer, pH 6.0; and (5) 10 mM NaCl. Fluorescence was measured using a Hitachi Perkin-Elmer spectrofluorometer, Model MPF-2A, with an excitation wavelength of 335 nm and an emission wavelength of 410 nm. The fluorescence was linear with enzyme concentration in square cross section cuvettes (1 cm $\times$ 1 cm) on the concentration range used. Fluorescence measurements were made at 37°C.

Enzyme activity was measured, in terms of fluorescence units per minute, by the rate of production of free NA. A standard solution of NA in the assay buffer was used to calibrate the fluorometer. The enzymatic activity on the entrapped enzyme was determined on samples withdrawn from the reaction mixture at different times during the course of the reaction in order to obtain more detailed kinetic measurements and to eliminate fluorescence scattering of the fibers. The samples were diluted with buffer for fluorescence measurements.

V_{max} and K_m values for free enzyme were determined from the initial rates using five to seven different substrate concentrations in Lineweaver–Burk plots. Satisfactory Michaelis–Menten kinetics were obtained with the least-squares method. The activity of DAP I is expressed as the percent ratio between the initial reaction rates of the same amount of free and entrapped enzyme (8). Before measuring the activity the fibers were washed and treated as described in the literature (6).

RESULTS AND DISCUSSION

Figure 1 shows the Lineweaver–Burk plot for unsupported bovine spleen DAP I, using Gly-Phe-NA as a substrate. A K_m and a V_{max} of

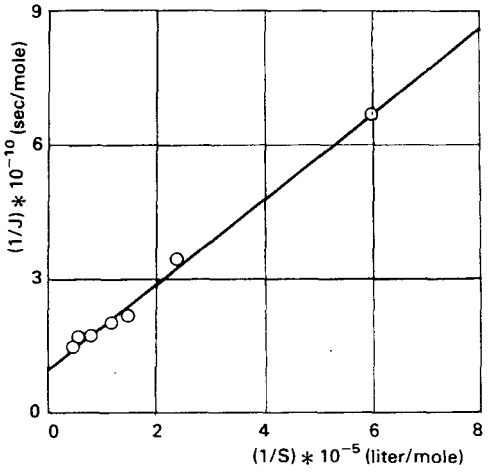

FIG. 1. Lineweaver–Burk plot for native bovine spleen DAP I with glycyl-L-phenylalanyl-β-naphthylamide.

0.098 ± 0.008 mM and $6.2 \pm 0.5 \times 10^{-3}$ μmol/min, respectively, were obtained. These values are slightly different from those reported in the literature for DAP I from rat liver (3). This discrepancy can be ascribed to the different origin of the enzyme and to experimental conditions.

Time versus concentration profiles have been determined for the immobilized enzyme at different stirrer speeds in order to evaluate the influence of external diffusional resistance. Figure 2 illustrates a correlation

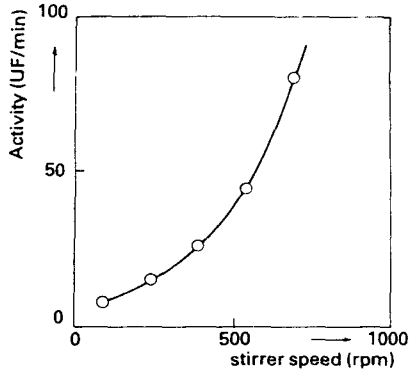

FIG. 2. Activity versus stirrer speed of cellulose fiber-entrapped DAP I.

between degree of magnetic agitation and enzymatic activity. Stirrer speeds higher than 700 rpm have also been tried; however, under these conditions, the fibers tend to agglomerate, thus affecting the reliability of the data. The use of a mechanical stirrer improved the system. The activity of the entrapped enzyme improved from 9% at maximum magnetic stirring, to 13% under mechanical stirring. These numbers are likely to contain, internal diffusion limitations in addition to external mass transfer contributions.

Moreover, Lineweaver-Burk plots for the entrapped enzyme did not give reproducible results. These findings suggest that the system is complicated by binding of NA to the cellulose matrix. In order to clarify this aspect, the amount of NA absorbed on the fibers before and after heat denaturation at 110°C has been determined. The results are reported in Fig. 3. However, a correction of the kinetic data for the absorption remained unsuccessful.

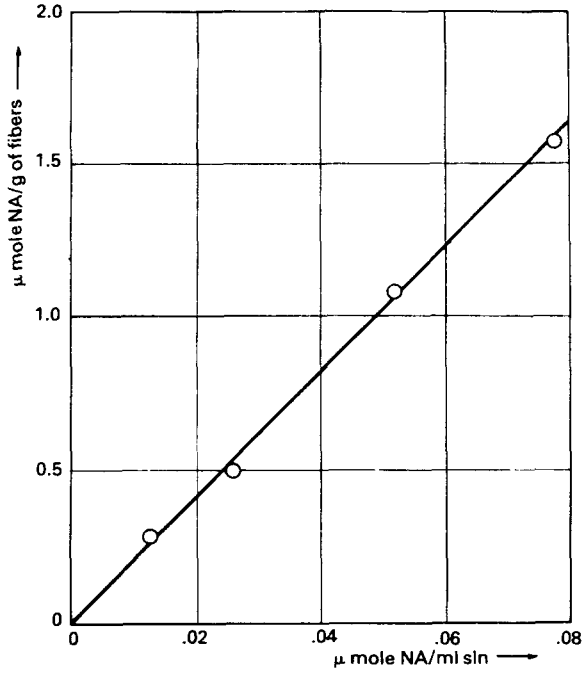

FIG. 3. Absorption isotherm at 37°C of β-naphthylamine on cellulose triacetate fibers.

CONCLUSIONS

DAP I can be immobilized on cellulose triacetate fibers following the usual procedures. The activity of the entrapped enxyme (13%) is sufficient for analytical purposes. Limitations to activity arise from intra- and inter-particle diffusion. Preliminary experiments indicate that some residual activity can be brought about by improving the mixing system, e.g., by the use of fixed-bed reactor.

The fact that NA is absorbed to the cellulose fibers indicates that a different substrate is needed for the determination of the actual activity of the entrapped enzyme. It also suggests that the determination of the influence on activity of parameters such as pH and ionic strength be postponed until a better assay system is found.

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