# ACTIVITY OF CARBOXYPEPTIDASE B WITH RESPECT TO THE CHROMOPHORIC SUBSTRATE 2,4-DINITROPHENYL-GLYCYL-GLYCYL-L-ARGININE

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For the colorimetric determination of the activity of carboxypeptidase B, it has been proposed to use the colored tripeptide 2,4-dinitrophenyl-glycyl-glycyl-L-arginine as substrate [1]. Its hydrolysis by carboxypeptidase B forms the colored product DNP-glycyl-glycine which, like the initial substrate, has its absorption maximum at 360 nm, but differs from the latter in its physicochemical properties. The method described previously for the quantitative separation of DNP-Gly-Gly and the uncleaved substrate is based on the difference in their solubilities in organic solvents [1].

We have simplified the separation of DNP-Gly-Gly from the uncleaved substrate by using their different behavior in the presence of a sulfonated cation-exchange resin due to the difference in the charges of the corresponding ions and of the sulfonated cation-exchange resin Dowex 50 × 4 (Serva, GFR). To stop hydrolysis, the incubation mixture was acidified to pH 2. Under these conditions the DNP-Gly-Gly-L-Arg is sorbed completely on Dowex 50 × 4 in 5 min. However, the partial sorption of DNP-Gly-Gly on the resin is also observed, but it was possible to suppress this by the addition of 50% propyl alcohol.

The results of the adsorption of DNP-Gly-Gly-L-Arg and of DNP-Gly-Gly on 2 g of Dowex 50 × 4 sulfonated cation-exchange resin (100-200 mesh) in 50% isopropanol at pH 2.0 are given below (in μmole):

| DNP-Gly-Gly in the sample | DNP-Gly-Gly in the filtrate | DNP-Gly-Gly-L-Arg in the sample | DNP-Gly-Gly-L-Arg in the filtrate |
|---|---|---|---|
| 0,163 | 0,160 | 0,336 | 0,036 |
| 0,326 | 0,308 | 0,673 | 0,053 |
| 0,490 | 0,460 | 1,010 | 0,083 |
| 0,653 | 0,600 | 1,346 | 0,086 |
| 0,816 | 0,733 | 1,683 | 0,106 |

The activity of the carboxypeptidase B was determined in the following way: to 0.3 ml of a solution of carboxypeptidase B in 0.05 M borate buffer, pH 8.0, was added 0.1 ml of a 2.5 mM solution of cobalt chloride, the mixture was kept at 20°C for 15 min [2], and then 2 ml of a 0.2 mM solution of DNP-Gly-Gly-L-Arg in 0.05 M borate buffer, pH 8.0 (optical density 3 at 360 nm) was added. The mixture was incubated at 37°C for 30 min, and then 0.1 ml of 1 N hydrochloric acid was added to pH 2.0 according to universal indicator. To each sample was added propyl alcohol (equal volume, 2.5 ml) and 2 g of moist Dowex 50 × 4 resin (100-200 mesh). Before use, the resin in the sodium form was equilibrated with 0.01 M citrate buffer, pH 2.0. The samples were shaken for 5 min on a shaking machine and were filtered through a paper filter, and the optical density was determined at 360 nm on a SF-4 spectrophotometer. A control experiment was performed under the same conditions without the addition of the enzyme.

A linear relationship between the amount of DNP-Gly-Gly formed and the concentration of the enzyme was preserved when the amount of carboxypeptidase B (Worthington, USA) in the sample was 0.8-2.4 μg.

The kinetic constants of the hydrolysis of DNP-Gly-Gly-L-Arg by carboxypeptidase B found at pH 8.0 and 37°C after incubation for 7 min were $K_M = 1.66 \cdot 10^{-3}$ M and $K_{cat} = 14$ sec$^{-1}$. For comparison, we may mention that $K_m$ and $K_{cat}$ are, respectively, $2.2 \cdot 10^{-4}$ M and 105 sec$^{-1}$ for hippuryl-L-argine, $1.5 \cdot 10^{-4}$ M and 87 sec$^{-1}$ for benzoyl-α-L-glutamyl-L-arginine, and $1.8 \cdot 10^{-4}$ M and 8.6 sec$^{-1}$ for α-N-benzoyl-

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L-lysyl-L-lysine [3]. Thus, the kinetic characteristics of DNP-Gly-Gly-L-Arg and the traditional substrates for carboxypeptidase are fully comparable. The proposed method of determining the activity of carboxypeptidase B does not require special apparatus and possesses considerable advantages in routine determinations. We assume that a similar method can be used for the determination of the activities of other enzymes.

## LITERATURE CITED


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