CONTINUOUS RESOLUTION OF ACYL DL-AMINO ACIDS BY CARRIER-BOUND KIDNEY ACYLASE. I.

T. BARTH and H. MAŠKOVÁ*

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague 6

Received July 23rd, 1970

Resolution of a racemic mixture of N-protected amino acid with the aid of a stereospecific acylase is a conventional method of organic chemistry. New impetus for the method appeared with the successful preparations of the so-called carrier-bound enzymes, *i.e.* water-insoluble enzymes. Tosa and coworkers[1-4] described a continuous resolution using bacterial acylase bound to DEAE-cellulose and DEAE-Sephadex. The substantially greater stability of the bound enzyme, the practically time-unlimited continuous reaction even at temperatures where the free enzyme denatures rapidly, predestined this method for industrial applications.

During the resolution of acetyl β-pyrrolo-DL-alanine with kidney acylase and by applying the usual insertion method we found that the deacetylation reaction is powerfully inhibited by the reaction product. Therefore, we prepared β-pyrrolo-L-alanine by continuous resolution which has the advantage of continuously removing the reaction products. We described the type of binding to the carrier and the results of continuous resolution of the amino acid as well as of acetyl DL-methionine which was used as model substrate.

EXPERIMENTAL

Preparation of acylase. Kidney acylase I was prepared from pig kidneys according to Birnbaum[5]. The resulting preparation was purified on a column of DEAE-cellulose[6] (activity 7 units/mg), dialyzed and freeze-dried. (One unit of activity is defined as the amount of enzyme catalyzing the transformation of 1 μmol substrate per min at 37°C.) The amount of deacetylated amino acid was determined by the ninhydrin test[7].

RESULTS

Binding of enzyme to carrier. DEAE-cellulose was transferred to the phosphate form and equilibrated with 0·01M sodium buffer at pH 7·0. After filling the column provided with a jacket, a solution of acylase I (4—10 mg/ml) is applied to the column until the column is saturated with the enzyme. Then the column is washed with 100 ml 0·01M sodium phosphate buffer at pH 7·0.

Continuous resolution of acetyl-DL-methionine. The column with the bound enzyme was washed with a solution of acetyl DL-methionine. The rate of deacetylation of substrate in dependence on its concentration, the pH of the solution used and the rate of flow was estimated. It was found that by decreasing the pH of the substrate solution below 6·6 or by increasing substrate concentration above 0·1M the enzyme is gradually eluted from the column. To determine the dependence of the rate of deacetylation of substrate on the rate of flow the following conditions were used: 0·02M solution of N-acetyl DL-methionine was adjusted to pH 7·0—7·1 with 2M-NaOH. The rate of flow was varied between 3 and 15 ml/h. Fig. 1 shows the results of the rate of splitting and the amount of deacetylated substrate on changing the rate of flow through the column. The reaction

* Graduate student of biochemistry, Dept. of Biochemistry, Charles University, Prague.

temperature was maintained at 37°C. In the course of 20 days of uninterrupted column resolution only a 20% decrease of activity of the bound enzyme was observed.

Continuous resolution of N-*acetyl*-β-*pyrrolo*-DL-*alanine.* Application of this type of resolution was found to be more suitable than deacetylation carried out by the insertion method since the decomposition products are being removed here continuously. If partially split substrate is recycled through the column the reaction product can be adsorbed on a cation exchanger placed in series with the deacetylation column. Detailed data on the isolation of β-pyrrolo-L-alanine were published elsewhere[8] and only principal features of the enzymic resolution are given here. The column of DEAE-cellulose (1 × 4 cm) after fixing the acylase was eluted at a rate of 3 ml/h with a solution containing 4·5 mg substrate/ml. The total amount of N-acetyl-β-pyrrolo-DL-alanine was 694 mg. The substrate solution before application to the column was adjusted with ammonium hydroxide to pH 7·0—7·1. The free amino acid was adsorbed to a cation exchanger and the filtrate was recycled through the column with acylase. The total yield of free amino acid was 48%.

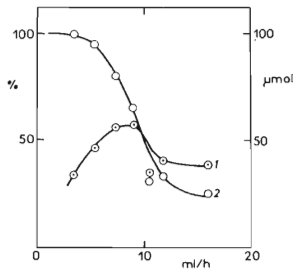


FIG. 1

Dependence of Deacetylation of N-Acetyl DL-methionine on the Rate of Flow of a 0·02M Substrate Solution through a Column of DEAE-Cellulose with Bound Acylase
○ Split N-acetyl-L-methionine in %, ⊙ total amount of deacetylated L-methionine in µmol. The rate of flow is given in ml/h.

DISCUSSION

Similarly to the case of microbial acylase, the kidney enzyme adsorbed on DEAE-cellulose maintains its enzyme activity. The character of the binding only restricts somewhat the possibilities of applying excessively high concentrations of substrate during continuous resolution. This disadvantage is compensated for by the stability of the fixed enzyme which makes it possible to conduct the reaction for several weeks.

The application of continuous resolution appears to be the only solution in cases where the enzyme is inhibited by the reaction products. Although it is frequently not possible on the basis of the ratio of splitting rate of a readily hydrolyzable substrate and the one under study to estimate the conditions for continuous resolution the example of N-acetyl-β-pyrrole-L-alanine shows that a fine yield may be obtained by repeated resolution. It will be attempted in the future to prepare an acylase bound by covalent bonds to a water-insoluble carrier.

REFERENCES

1. Tosa T., Mori T., Fuse N., Chibata I.: Enzymology *31*, 15 (1966).
2. Tosa T., Mori T., Fuse N., Chibata I.: Enzymology *31*, 214 (1966).

3. Tosa T., Mori T., Fuse N., Chibata I.: Enzymology *32*, 154 (1967).
4. Tosa T., Mori T., Fuse N., Chibata I.: Biotechnol. Bioengin. *9*, 603 (1967).
5. Birnbaum S. M. in the book: *Methods in Enzymology* (S. P. Collowick, N. O. Kaplan, Eds.), Vol. II, p. 115. Academic Press, New York 1964.
6. Mašková H.: Unpublished results.
7. Moore S., Stein W. H.: J. Biol. Chem. *176*, 367 (1948).
8. Poduška K., Rudinger J., Gloede J., Gross H.: This Journal *34*, 630 (1969).

Translated by A. Kotyk.

BINDING OF BENACTYZIN TO BOVINE SERUM ALBUMIN

J. Bajgar and J. Patočka

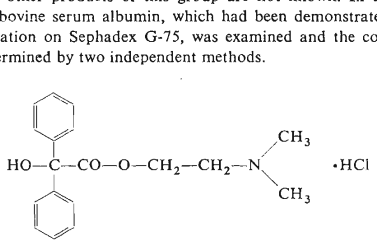
J. E. Purkyně Mtlitary Medical Institute of Research and Postgraduate Training, Hradec Králové

Received June 17th, 1970

Many of the compounds used as drugs are bound in the organism to proteins, predominantly to serum albumin[1]. The character of this binding affects their transport, distribution, and elimination. Simultaneously the binding of these compounds to proteins can be utilized as a model system representing the interaction of the drug with the receptor.

In their review, Mayer and Guttman[1] discussed methods, interpretation of experimental results, and compounds which have been studied with regard to their binding to proteins. This review shows that the group of parasympaticolytics has been studied relatively very little, actually only one of its members – atropine. Oroszlan and Maengwyn-Davies[2–4] have demonstrated the binding of atropine to bovine serum albumin, determined the pharmacologic effect of the atropine–albumin complex, and some of the constants characterizing this binding.

Similar studies on other products of this group are not known. In this study the binding of benactyzin (*I*) to bovine serum albumin, which had been demonstrated[5] in preliminary experiments by gel filtration on Sephadex G-75, was examined and the constants characterizing this binding were determined by two independent methods.



I