Stereospecificity of Allantoin Degradation in *Streptococcus allantoicus*

Previous studies dealt with the intermediates formed during hydrolysis of allantoin in various microorganisms[1-4]. In this report the stereospecificity of the enzymes and the optical form of the intermediates involved in the anaerobic allantoin degradation of *Streptococcus allantoicus* is communicated.

Materials and methods. Allantoinase and allantoate amidohydrolase were purified from cell-free extracts of *S. allantoicus* as described before[4,5]. Partially purified ureidoglycolase, free from allantoinase and allantoate amidohydrolase, was obtained by gradient elution of the cell-free extract bound to DEAE-cellulose at pH 7.5. The fraction emerging from the column between 0.23–0.28 *M Tris*-chloride buffer (pH 7.5) was used in the enzymatic studies. The specific activity of this material was 2.6 U/mg protein. 1 U of activity is defined as the amount of enzyme which releases 1 μM of glyoxylate per min at 30 °C. Enzymatic activities were determined by measuring the product formed according to a differential glyoxylate analysis[2]. Optical rotation was measured in a 10 cm tube in a Perkin-Elmer polarimeter, model 141, with a sodium vapor lamp.

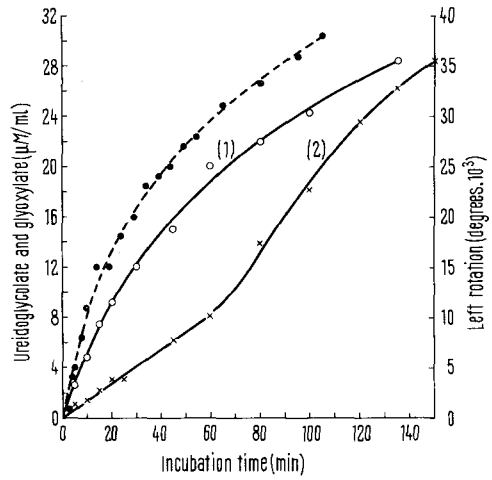

Fig. 1. Formation of (—)-ureidoglycolate during allantoate hydrolysis by allantoate amidohydrolase. Enzyme (24 μg protein) was activated as described previously[5]. The amounts of ureidoglycolate (1) and glyoxylate (2) and the change of optical rotation were determined.

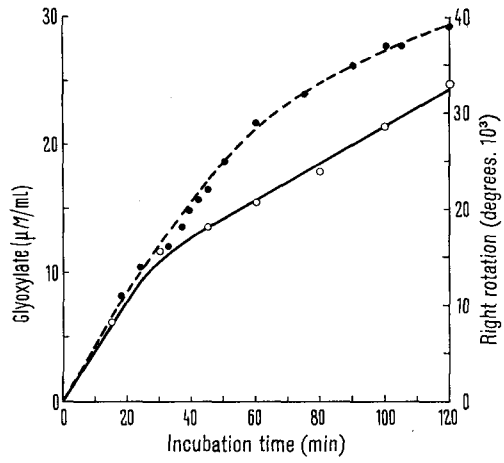

Fig. 2. Degradation of (—)-ureidoglycolate by ureidoglycolase. Glyoxylate production and the change of optical rotation were measured. Correction was made for the non-enzymic cleavage of ureidoglycolate.

Results and discussion. Allantoin degradation to glyoxylate in anaerobic bacteria is catalyzed by allantoinase, allantoate amidohydrolase and ureidoglycolase. Allantoin contains an asymmetrical carbon atom, but allantoinase which transforms allantoin to allantoate appeared to be aspecific[4]. Aspecificity was not the result of the presence of an allantoin racemase[6]. Degradation of allantoate, itself a symmetrical molecule, to ureidoglycolate by allantoate amidohydrolase was accompanied by an increase of left rotation, which paralleled the amount of ureidoglycolate formed (Figure 1). Ureidoglycolate accounted for the increase of left rotation, since calculation of $[\alpha]_D^{30}$ from the amount of ureidoglycolate formed and the observed change of optical rotation revealed the value $-10.5° \pm 1°$ which was in good agreement with that determined in other experiments[2,3]. (—)-Ureidoglycolate formed by the action of allantoate amidohydrolase was broken down by ureidoglycolase, because incubation of racemic ureidoglycolate with this enzyme at pH 8.2, which was the optimal pH, resulted in a change of optical rotation in the right direction (Figure 2). For (+)-ureidoglycolate an $[\alpha]_D^{30} = +10.5° \pm 1°$ was calculated. Therefore, (—)-ureidoglycolate is the real intermediate during allantoin degradation in *S. allantoicus* and is further converted to glyoxylate and urea by (—)-ureidoglycolase.

In contrast to these results, *Pseudomonas acidovorans* degrades allantoin by means of (+)-allantoinase[4], an allantoate amidohydrolase which produces (+)-ureidoglycolate and (+)-ureidoglycolase[2].

The absolute configuration of (+)- and (—)-ureidoglycolate is not known at present.

Zusammenfassung. Die Stereospezifität der Enzyme des Allantoinabbaus in *Streptococcus allantoicus* und die optische Drehung der Reaktionsprodukte wurde bestimmt. Allantoinase war aspezifisch, während Allantoate-amidohydrolase ausschliesslich (—)-Ureidoglykolate bildete. Diese Substanz wurde durch (—)-Ureidoglykolase hydrolysiert.

C. VAN DER DRIFT and G. D. VOGELS

University of Nijmegen, Department of Biochemistry, Nijmegen (The Netherlands), 20 December 1968.

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