BBA 61196

Isolation and purification of the isocitrate dehydrogenase ($NADP^+$) of *Azotobacter vinelandii*

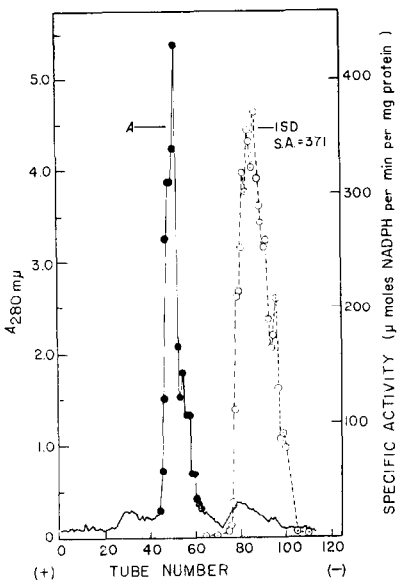
Cell-free extracts of *Azotobacter vinelandii* strain O (AV-O) possess an unusually active isocitrate dehydrogenase (*threo*-D_s-isocitrate:$NADP^+$ oxidoreductase (decarboxylating), EC 1.1.1.42)[1]. Comparative studies on this enzyme, using other strains of *A. vinelandii*, as well as a nonnitrogen-fixing mutant (AV-3), indicated that the exceptionally high specific activity may be related to the metabolic requirements of nitrogen fixation. In the process of investigating this possibility, a relatively simple electrophoretic procedure was developed for the isolation and purification of the isocitrate dehydrogenase of *A. vinelandii*. This procedure is described in this communication.

Analyses of the purified enzyme by polyacrylamide disc electrophoresis showed a single protein band which corresponded to the enzyme activity band. Specific activities of over 300 μmoles of $NADP^+$ reduced per min per mg protein at 37° were obtained. Activities of this order of magnitude have never been reported for either the bacterial or mammalian isocitrate dehydrogenases.

A. vinelandii strain O cells were grown under nitrogen-fixing conditions on a modified Burk's liquid medium[2] using 1% sodium acetate as sole carbon source. Resting cells were washed, standardized turbidimetrically and disrupted by sonic oscillation. The enzyme was found in the clarified supernatant fraction, or "S_3" after centrifugation at 144 000 $\times g$ for 2 h (ref. 3). The isocitrate dehydrogenase concentrated in the precipitate collected between the 70 and 80% $(NH_4)_2SO_4$ saturation level. This fraction is referred to as the S_3 (70–80) fraction.

Further purification was achieved with the Canalco Model 6 and Prep-Disc gel electrophoretic systems using Tris–glycine buffer (pH 8.8). Prior to electrophoresis, the S_3 (70–80) fraction was dialyzed against 0.01 M phosphate buffer (pH 7.5) to remove all residual NH_4^+. All electrolyte buffers contained 0.5 mM sodium isocitrate and the elution buffer used in the Prep-Disc system also contained 20 μM $MnCl_2$. These additions prevented the inactivation of the isocitrate dehydrogenase. The fractions collected from the preparatory disc electrophoresis column were monitored for protein content by absorbance measurements at 280 mμ. Analyses for isocitrate dehydrogenase activity were performed measuring $NADP^+$ reduction as previously described[1]. The gel assay used for the isocitrate dehydrogenase was a modification of that reported by GOLDBERG[4] for the lactate dehydrogenase. The current used for electrophoresis was 4 mA per gel for the analytical gels and 5 mA for the Prep-Disc column.

Fig. 1 shows the elution pattern obtained by electrophoresis on a 5% acrylamide separation gel in the Prep-Disc column. The sample used was an S_3 (70–80) fraction which contained 36 mg of protein. After elution the peak activity fraction had a specific activity of 371 μmoles of $NADP^+$ reduced per min per mg protein at 37° and five other fractions had specific activities of over 300. These six fractions contained a total protein concentration of 2 mg. The specific activity of the parent S_3 fraction was 11.2, that of the S_3 (70–80) fraction was 143. A 33-fold purification of the enzyme was attained by this procedure and recovery of activity units was 55%.



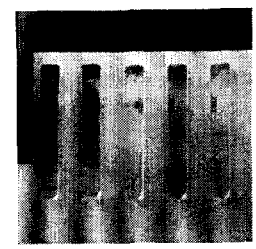


Fig. 1. Elution pattern for Prep-Disc electrophoresis of the S_3 (70–80) fraction of *A. vinelandii* using 5% acrylamide gel. Absorbance at 280 mμ and specific activity for the isocitrate dehydrogenase (ISD) are plotted as a function of the volume of eluate.

Fig. 2. Analytical disc electrophoresis of the various fractions obtained during the purification of the isocitrate dehydrogenase. (A) Protein stain of the parent S_3 fraction. (B) Protein stain of the S_3 (70–80) $(NH_4)_2SO_4$ fraction. (C) Isocitrate dehydrogenase assay stain of the S_3 (70–80) fraction. (D) Protein stain of the purified Prep-Disc peak activity fraction. (E) Isocitrate dehydrogenase assay stain of the purified Prep-Disc peak activity fraction.

The various enzyme fractions obtained by this procedure were monitored by 10% acrylamide analytical disc electrophoresis (Fig. 2). The first gel (A) represents the protein analysis for the parent S_3 fraction while Gel B is the protein pattern for the S_3 (70–80) fraction. This latter sample was used for the isolation of the isocitrate dehydrogenase by the Prep-Disc experiment shown in Fig. 1. This fraction contained at least 11 protein bands. Gel C is the corresponding isocitrate dehydrogenase assay pattern for Gel B which shows the location of the active enzyme band. Gels D and E represent the analyses of the peak activity fraction shown in Fig. 1. Only one protein band was visible and it corresponded precisely with the isocitrate dehydrogenase activity band. The intensely stained protein band in Gel D resulted from the fact that a large amount of the purified enzyme (300 μg protein) was added to the analytical disc system to determine whether other contaminating proteins were present. The difference noted in the position of the protein and activity bands, in Gels D and E, was due to a slightly longer electrophoresis time (1 h) as compared to that used for Gels B and C which were electrophoresed for 45 min.

This investigation was supported by the U.S. Public Health Service grant GM-12279 from the National Institute of General Medical Sciences. One of the authors (C.R.B.) is a National Institutes of Health Predoctoral Trainee.

Department of Microbiology,
University of Texas at Austin,
Austin, Texas 78712 (U.S.A.)

C. R. BARRERA
PETER JURTSHUK

1 P. JURTSHUK, C. R. BARRERA AND S. MANNING, *Can. J. Microbiol.*, 15 (1969) 321.
2 J. T. DUFF AND O. WYSS, *J. Gen. Microbiol.*, 24 (1961) 273.
3 P. JURTSHUK, S. MANNING AND C. R. BARRERA, *Can. J. Microbiol.*, 14 (1968) 775.
4 E. GOLDBERG, *Science*, 139 (1963) 602.

Received June 2nd, 1969

Biochim. Biophys. Acta, 191 (1969) 193–195

BBA 61197

Ontogenesis of microsomal ATPase in the rabbit kidney

The ability to concentrate urine is related to the build-up of an osmotic gradient along the renal medulla[1]. This osmotic gradient depends on a constant supply of Na^+ which is actively transported by epithelial cells along the nephron[2,3]. Thus, Na^+ deprivation results in a loss of concentrating ability[4].

The concentrating function of the kidney is poorly developed at birth and its full capacity is achieved only during extra-uterine development[5,6]. On the other hand, activity of a specific microsomal enzyme in the kidney, (Na^+, K^+)-dependent ATPase (ATP phosphohydrolase, EC 3.6.1.3), has been shown to be related to Na^+ reabsorption in the renal tubules[7]. The activity of this enzyme in the kidney is regulated by mineralocorticoids[8,9] and by Na^+ intake[10]. It seemed of interest, therefore, to study the ontogenetic development of this enzyme in the kidney during fetal development and after delivery, in relation to the maturation of the concentrating mechanism.

We have, therefore, investigated microsomal ATPase in kidneys of rabbit fetuses (28th day of gestation), on the day of birth, at the age of 10 days and in adult rabbits. Kidney tissue (approx. 1 g) was homogenized in 10 vol. of 0.25 M sucrose

TABLE I

ONTOGENESIS OF MICROSOMAL ATPASE IN THE RABBIT KIDNEY

Activities are expressed as mg P_i liberated per mg microsomal protein per h. n.s., difference between two consecutive stages of growth not significant.

Age	*Number of experiments*	*Mg^{2+}-ATPase activity*		*(Na^+, K^+)-dependent ATPase activity*	
Fetus	9	3.17 ± 0.17	$P < 0.01$	0.81 ± 0.13	n.s.
1 day	7	4.23 ± 0.07	$P < 0.001$	1.15 ± 0.24	n.s.
10 days	4	7.94 ± 0.17	$P < 0.05$	0.62 ± 0.24	$P < 0.01$
Adult	7	8.98 ± 0.27		2.05 ± 0.25	