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Multiple Forms of Rat Kidney Mutarotase

Four forms of mutarotase [EC 5.1.3.3] from rat kidney were demonstrated by diethylaminoethyl-cellulose column chromatography. A major form of them was purified to homogeneity.

Mutarotase [aldose 1-epimerase, EC 5.1.3.3] catalyzes the interconversion of the α- and β-anomers of D-glucose and other sugars. Any definite function of this enzyme has not yet been clarified, but it has been postulated that the enzyme may be involved in the transport of sugars[1] or in D-glucose metabolism.[2] Purification of the enzyme has been reported from the kidney of various mammals, *e.g.* hog,[3,4] beef,[5] sheep, lamb, and rabbit,[6] but not from the kidney of rat. In the present paper, we describe that the four forms of rat kidney mutarotase were demonstrated by diethylaminoethyl (DEAE)-cellulose column chromatography, that their isoelectric points were distinctive, and that the major form (type II) was purified to homogeneity.

Mutarotase activity was determined by the method of Miwa and Okuda.[7] Isoelectric focusing was performed according to the instructions of the manufacturer using a 110 ml column model 8101 of LKB (Sweden). The molecular weight of the purified mutarotase was determined by polyacrylamide gel electrophoresis in sodium dodecyl sulfate according to the method of Weber and Osborn.[8] Protein was determined by the method of Lowry, *et al.*[9]

Unless noted otherwise, all purification procedures were carried out at 4°. The whole kidney (400 g) of rat (Wistar strain) was homogenized with 400 ml of 0.1M Tris–HCl buffer (pH 7.2) and 160 ml of chloroform in a Waring blender for 3 min at room temperature. This homogenization with chloroform decreased slightly the recovery of mutarotase compared with that without chloroform, but fairly simplified the subsequent purification procedure. The homogenate was centrifuged and the supernatant was collected. After ammonium sulfate fractionation of the supernatant, the resultant precipitate (between 25 and 52% saturation) was dissolved in 10 mM Tris–HCl buffer (pH 7.2) and dialyzed against the same buffer. The impermeate was centrifuged and the supernatant obtained was concentrated by ultrafiltration to a final volume of about 45 ml and applied to a column (4.5×70 cm) of Sephadex G-75 equilibrated with 10 mM Tris–HCl buffer (pH 7.2). The effluent containing mutarotase activity was concentrated to a final volume of 5 ml and applied to a column (2.5×25 cm) of DEAE-cellulose (Whatman, DE 52), which was previously equilibrated with 10 mM Tris–HCl buffer (pH 7.2). By elution with 1.2 liter of linear Tris–HCl buffer gradient (10 to 120 mM, pH 7.2), four peaks with mutarotase activity were observed (designated Type I, II, III, and IV in order of elution). The fractions containing mutarotase Type II were concentrated and dialyzed against 5 mM sodium phosphate buffer (pH 7.2). The dialyzed sample was applied to a column (2.5×20 cm) of a mixture (2: 1, w/w) of hydroxylapatite (Bio–Rad, Bio–Gel HTP) and cellulose powder (Toyo Kagaku, type A), which was previously equilibrated with the same buffer. By elution with 600 ml of linear sodium phosphate buffer gradient (5 to 70 mM, pH

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7.2), pure mutarotase type II was obtained in the effluent with a phosphate ion concentration of about 10 mM.

The elution pattern of rat kidney mutarotase from the DEAE-cellulose column seems to show that four different forms of mutarotase exist in rat kidney (Fig. 1). In order to exclude the possibility that this multiplicity may be due to artifacts formed during the purification procedures as known in the case of multiple forms of glucosephosphate isomerase, which resulted from the oxidation of the sulfhydryl groups,[10] the purification procedures of rat kidney mutarotase were temporarily modified as follows: addition of 5 mM β-mercaptoethanol in all steps; homogenization of rat kidney with 0.1M Tris–HCl buffer (pH 7.2) (avoidance of use of chloroform); elimination of ammonium sulfate fractionation. After a series of modifications, Sephadex G-75 and DEAE-cellulose column chromatographies were performed in the same manner as the original method in the presence of 5 mM β-mercaptoethanol. Rechromatography of each of mutarotase type I and II, two major forms, on DEAE-cellulose column did not result in the formation of any other forms. Thus, it is believable that four forms of mutarotase exist in the rat kidney.

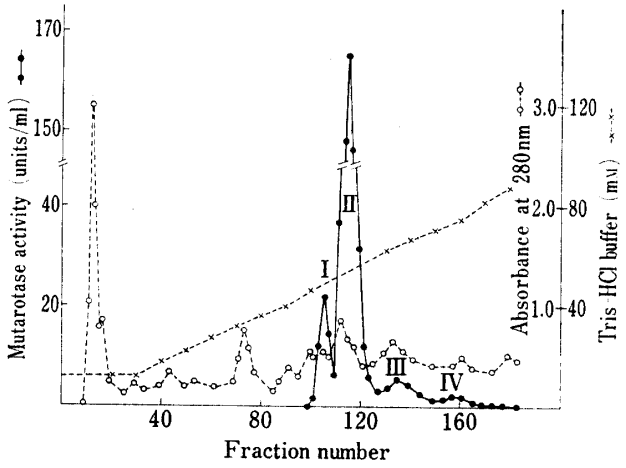

Fig. 1. DEAE-cellulose Column Chromatography of Rat Kidney Mutarotase

flow rate: 25 ml/hr
fraction volume: 5 ml

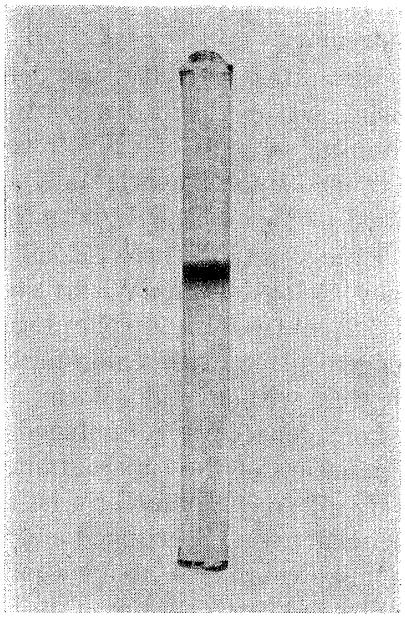

Fig. 2. Polyacrylamide Disc Gel Electrophoresis of Purified Rat Kidney Mutarotase Type II

Isoelectric focusing electrophoresis (pH 4—6) of each of the four forms revealed that they have distinctive isoelectric points (pH 5.56, 5.29, 5.24, and 5.04 in order of type I, II, III, and IV) which are compatible with the order of elution from the DEAE-cellulose column. Many reports on the purification of mutarotase from a bacterium (*E. coli*),[11] a mold (*P. notatum*),[12] higher plants,[13] and the kidney and liver of various mammals, including hog,[3,4] beef,[5] sheep, lamb, and rabbit,[6] but not rat, have been published, but the presence of multiple forms of the enzyme has not yet been reported. To know whether or not the multiplicity is observed only in rat kidney, purification of the enzyme from hog kidney cortex and rat liver was also achieved, and it was found that two forms corresponding to type I and II of rat kidney mutarotase in the elution pattern on DEAE-cellulose column chromatography

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were present in hog kidney cortex and three forms corresponding to type I, II, and III in rat liver. Therefore, the multiplicity of mutarotase appears to be widely observed in various species and various organs of mammals.

Since it has been suggested that mutarotase may play some role in reabsorption of D-glucose at the proximal tubules of kidney cortex,[14,15] we compared the relative proportion of multiple forms of mutarotase from rat whole kidney with that from kidney cortex. The relative activities of four forms (type I, II, III, and IV) of mutarotase in whole kidney were 11, 80, 7, and 2% of the total activity as shown in Fig. 1, and those in kidney cortex were almost the same except for a slight decrease of the activity of mutarotase type III. So, it seems that any of four forms does not specifically localize only in kidney cortex. Moreover, we found that the mutarotase activity (45 units[7]/g wet tissue) in kidney cortex was almost the same as that in whole kidney in contradiction to the report[16] of Bailey, *et al.*, in which they described that the mutarotase activity ratio of kidney cortex to whole kidney was about 2.0.

By applying crude mutarotase type II (obtained by DEAE-cellulose column chromatography) on a hydroxylapatite column, it was purified about 540-fold with a recovery of 25% from homogenate supernatant. The purified enzyme was ascertained to be homogeneous by polyacrylamide disc gel electrophoresis (Fig. 2). The specific activity of pure mutarotase type II was 291 units per mg of protein. The optimal pH with 0.02 M EDTA buffer was 7.4. The *K*m value for α-D-glucose at pH 7.4 was determined to be 35 mM by the polarimetric method of Bailey, *et al.*[17] The molecular weight determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis was 40700. These physicochemical properties of pure mutarotase type II were fairly similar to those of other mammalian mutarotases reported by other workers.[3-6]

We found previously that the kidney mutarotase may appear in urine of patients[18] and rats[19] with nephrotic syndrome. We are now studying which form of mutarotase appears in urine.

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