HUMAN KIDNEY CARBONIC ANHYDRASES ELECTROPHORETIC DISTRIBUTION

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During the past few yers, carbonate hydro-lyase from animal and human erythrocytes (EC 4·2.1.1) and its various isozyme forms[1-5] have been extensively characterized. By contrast, very little is known about the chemical properties of carbonate hydro-lyases from organs. Of the many physiological functions ascribed to this enzyme the one which seems to be proved beyond doubt is its participation in the metabolism of inorganic ions in the kidney[6]. Our knowledge of kidney carbonate hydro-lyase, however, is far behind the great number of physiological and pharmaceutical reports. The kidney carbonate hydro-lyase from dog has been partly characterized by chromatography on DEAE-cellulose and by electrophoresis in polyacrylamide gel. In the cytoplasmatic portion of the kidney homogenate one main component, resembling electrophoretically the enzyme from dog erythrocytes, and one minor component were found[7]. The other part of kidney activity, contained in mitochodria, differed slightly from these two components by a lower sensitivity to inhibition by acetazolamide[8]. Analogous data on human kidney carbonate hydro-lyase has not been reported.

In preliminary experiments designed at the isolation of carbonate hydro-lyase from human kidney we found, according to our expectation, that a part of the activity belonged to isozymes from erythrocytes which remained in the tissue *post mortem*. From these isozymes differ in electrophoretic mobility and immunchemical specificity one (in agar gel) or two (in starch gel) components which obviously originate in the kidney tissue. The activity of these components is inhibited by acetazolamide.

Methods

Human kidneys obtained from autopsies were processed in 5—50 g batches. The frozen tissue was finely cut to slices and triturated intensively in the cold with sea sand (Merck) under continuous addition of 4 volumes of water. After 20 min ammonium sulfate was added to 0·6 saturation. A part of the inactive proteins was precipitated at this concentration while most of the activity remained in solution. After several hours of standing in the cold the solution was centrifuged in a cooled centrifuge (Janetzki, type K 23, 2800 g) and the supernatant desalted on Sephadex G-50 (column volume 270 ml, column diameter 28 mm). The activity of effluent fractions (10 ml) was determined. The part of the effluent which showed enzymatic activity was concentrated to approximately one tenth of the volume of the original homogenate by dialysis against an approximately ten-fold volume of 15% dextran solution. This solution was used in subsequent studies.

In one representative experiment (No N 5), 5·5 g of the kidney tissue (total activity 5500 Roughton units[9] and specific activity 22·5 units/mg protein) afforded a final solution of total activity 4800 units and specific activity 96 units. The yield of the activity was therefore 87% and the enrichment was 4-fold.

The zones of carbonate hydro-lyase after electrophoresis in agar- and starch-gel were detected by a method described earlier[10] based on the faster change of the indicator colour (bromthymol

blue infiltered into the gel) at those sites where the medium becomes more rapidly acidic as a result of the presence of the enzyme if the plate is exposed to CO_2 atmosphere. The sensitivity of this enzymatic reaction exceeds the sensitivity of staining by amido black; active zones can thus be detected in places where no zone is revealed by the subsequent detection of proteins.

The crude preparation of erythrocytic isozymes was prepared by precipitation of hemolyzed erythrocytes by ethanol–chloroform (preparation designated as EC-enzyme) in the usual manner[11] from fresh erythromass supplied by a blood bank. In the experiments described here the EC enzyme was used after ethanol had been dialyzed off and the enzyme solution slightly concentrated by dialysis against the dextran solution. The standard composition of the crude EC preparations is presented in Fig. 1 which shows a typical elution profile of activity and proteins on DEAE-Sephadex. The lyophilized EC enzyme (120 mg) was fractionated on a 35 . 2·7 cm column of DEAE-Sephadex A 50 equilibrated with 0·05M Tris-0·012M-HCl buffer and eluted with the same buffer. The effluent was collected in 10 ml fractions until both activity peaks emerged in the order isozyme C-isozyme B; the ionic strength was then increased by the addition of 0·2M-NaCl to the same buffer and isozyme A was collected. The method has been described in detail elsewhere[11].

The antisera to C and B isozyme antigens were prepared by immunization of rabbits with the EC enzyme. In the experiments described here, antiserum RACA No 4 was used. The latter was prepared by intramuscular immunization of the rabbit by a total dose of 110 mg of the EC enzyme with simultaneous sensibilization with freshly precipitated aluminum hydroxide in three doses administered over a period of 10 weeks. The antiserum employed was tested for the absence of antibodies to serum proteins and hemoglobin. The immunochemical specificity of the antiserum is demonstrated in Fig. 2* showing that all the precipitation lines after the immunoelectrophoresis of the EC enzyme, isozyme C, and isozyme B disappear after this antiserum has been saturated with pure fraction C or B, which had been obtained by lyophilization of the corresponding portions of the effluent from the separation of the EC enzyme on DEAE-Sephadex as described above. The result accords with the known fact that all the so far known subcomponents of the isozymes of erythrocytic carbonate hydro-lyase show immunospecificity of the C or B-type[3,5].

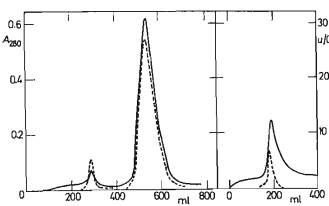

FIG. 1

Fractionation of EC Enzyme on DEAE-Sephadex A 50

Full line, absorbance of effluent at 280 nm in 0·5 cm cell of Zeiss VSU 1 spectrophotometer, broken line, relative activity determined by the modified flask method (ref.[9]). The second part of the curve represents elution after the addition of 0·2M-NaCl.

* See insert facing p. 2748.

RESULTS AND DISCUSSION

Fig. 3* shows the electrophoretic behaviour of the EC preparation, of the hemolysate of human erythrocytes, and of two final kidney extracts. The EC enzyme displays the presence of four zones of enzymatic activity. These are isozymes C, B, and A in order of increasing mobility. This order is determined unambiguously by known data on the electrophoretic mobility of these components[11]. The existence of the fourth, fastest component has been described in our preceding report[12] as an enzyme component detectable by this method always in the EC preparations and almost always in native hemolysates of human erythrocytes. Funakoshi and Deutsch[5] isolated later from a large amount of erythrocytes a number of proteins in crystalline state, which show the activity of carbonate hydro-lyases, and determined among others also their relative concentrations and electrophoretic mobilities. With respect to the data of these authors it is highly probable that our fourth component is identical with their isozyme D. This conclusion is evidenced by the fact that of the remaining isozymes which might be considered (*i.e.* E + F, O + P, G + H, and M + N) isozyme D is present in the highest concentration and moves always ahead of A on various supports.**

The hemolysate contains isozymes C, B, and A. The fourth component is in the Tris-buffer employed overlapped by the zone of hemoglobin. Two kidney preparations (No N 4 and N 5) contain three activity zones, which show the same electrophoretic mobility as the components of the EC enzyme and of the hemolysate. In addition two zones are present; one of them moves between isozyme A and D, the other one moves considerably faster and its activity is associated with detectable concentration of protein. Our results lead us to assume that the three slower components of the kidney preparation originate in the blood present in the organ. In this respect we are not suprised by the absence of isozyme D since the amount of blood present in the samples is substantially lower than would correspond to the concentration of the hemolysate sample, as indicated by the ratio of the intensities of the hemoglobin zones.

The activity of all zones, which have been detected in the starch gel, can be inhibited by acetazolamide and they represent therefore true carbonate hydro-lyases.

When subjected to agar-gel immunoelectrophoresis (Fig. 4*) with rabbit antiserum to the EC enzyme, both kidney preparations give precipitation lines at those sites where lines are also formed in experiments with the EC enzyme and the dilute hemolysate. These lines coincide with the localization of enzymatic activity as determined and marked before the immunoprecipitation reaction. This data together with the results of starch-gel electrophoresis and also the presence of hemoglobin in the samples of kidney extracts lead us to the justified conclusion that these components have their origin in the contamination of the tissue with blood. We found moreover in the anodic part of the pattern an active zone which does not precipitate with the antiserum used. This component we regard as kidney carbonate hydro-lyase. As to the slower component observed in the starch-gel electrophoresis pattern, its mobility is so close to that of the erythrocytic enzymes that the component may most likely belong to these enzymes.

By starch-gel electrophoresis and agar-gel immunoelectrophoresis kidney carbonate hydro-lyase was distinguished from the accompanying erythrocytic isozymes. Carbonate hydro-lyase, which has been regarded as an enzyme specific of the kidney tissue, migrates in starch gel as two active zones; one of the latter shows a considerably higher mobility than the erythrocytic isozymes

* See insert facing p. 2748.

** The employed detection method does not permit a direct determination of the concentration of individual components since it is based on an enzymatic reaction at a concentration of the EC enzyme chosen so low that it lies below the sensitivity limit of the staining procedure for proteins.

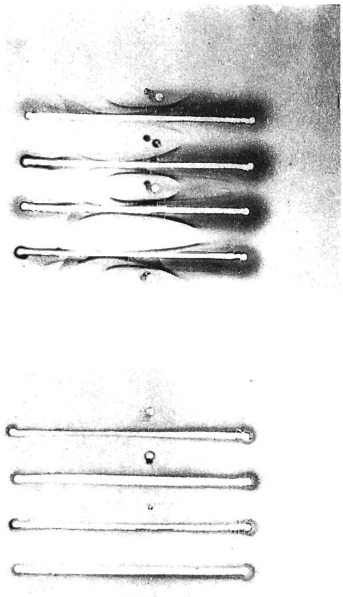

FIG. 2

Immunoelectrophoresis of Erythrocytic Isozymes of Carbonate Hydro-Lyase

a In path 3 and 5 (top) samples of the EC enzyme were applied, in path 2 and 3 isozymes C and B respectively obtained by lyophilization of the corresponding effluent fractions after fractionation of the EC enzyme on DEAE-Sephadex. The concentration is in all cases 5%. In the wells is rabbit antiserum to the EC enzyme RACA No 4 (7%)

Electrophoresis in agar gel Sevac: 100 V, 15 mA, 3 h in Na-barbiturate–barbituric acid buffer, pH 8·6, ionic strength 0·12.

b analogous experiment with antiserum RACA No 4 which had been saturated with a mixture of pure isozymes C and B obtained as described in the text.

Human Kidney Carbonic Anhydrases Electrophoretic Distribution

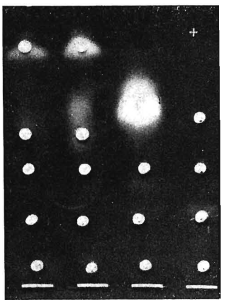

FIG. 3

Starch-Gel Electrophoresis of Carbonate Hydro-Lyases from Kidney and Erythrocytes

Samples from the left: EC enzyme, hemolysate of erythrocytes 1 : 9, preparations N 5 and N 4 from kidney. Electrophoresis: 100 V, 10 mA, 16 h in Poulik Tris-citrate buffer[13] using the vertical arrangement and the apparatus of Smithies[14].

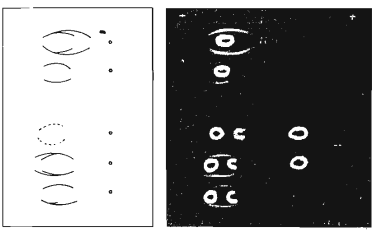

FIG. 4

Immunoelectrophoretic Pattern of Erythrocytic and Kidney Carbonate Hydro-Lyases

From top to bottom: *1*, EC enzyme; *2* portion of effluent with activity, obtained by desalting on Sephadex G-50, before concentration (see text); *3* inactive fraction of effluent from the same experiment; *4—5*, kidney preparations N 4 and N 5; *6*, dilute hemolysate. Electrophoresis 90 V, 8 mA, 5 h. Otherwise conditions the same as those described in legend to Fig. 2.

whereas the other one lies between the zones of isozymes A and D. In agar gel the faster kidney component can be found in the anodic region while the slower component most likely overlaps the erythrocytic isozymes in the cathodic region of the electrophoretic pattern. The anodic component gives a negative precipitation reaction with the antiserum to erythrocytic isozymes.

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