DISSOCIATION OF UREASE IN AQUEOUS 1,2-ETHANEDIOL

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SUMMARY

The addition of 1,2-ethanediol (33-50% v/v) to solutions of urease in pH 7 phosphate or pH 9 TRIS buffer causes dissociation of the molecules, M.W. 480,000, into halves. The dissociation has been demonstrated and its rate measured by means of acrylamide-gel electrophoresis as well as ultracentrifugal analysis. The half-life time for the process is about 13 hr in 33% glycol, 13 min in 50% glycol. The process may be reversed at pH 7, but not at pH 9, by diluting the medium with buffer to a glycol concentration of 10%.

INTRODUCTION

The molecules of many enzymes and other proteins contain two or more polypeptide chains (1,2). Considerable interest attaches to the question of what forces hold the chains together and how the chains may interact. Information concerning these matters may be gleaned from consideration of the treatments that cause the chains to separate and of the structural rearrangements that may concomitantly take place. Strong denaturants, such as concentrated guanidinium chloride, are the most generally effective dissociating agents, but they also tend to disrupt the internal structure of the subunits. Milder means, such as changing the pH and/or the surrounding salt concentration, are effective in some cases (2). The present communication deals with the dissociation of urease, induced by the addition of 1,2-ethanediol.

Urease exhibits an interesting behavior with respect to dissociation. Sedimentation velocity and equilibrium measurements at concentrations of 1-0.01% in pH 7 phosphate buffer correspond to a M.W. of 480,000 (3,4). In 6M guanidinium chloride, a much smaller value is obtained, 83,000 (5). Treatment with sufficient sodium dodecyl sulfate also results in a drastic

reduction of the weight, that was estimated to be in the range 50,000-80,000 (6,7); the product of SDS treatment gives a single band on acrylamide-gel electrophoresis (7). Very recently it has been reported that the enzyme contains but a single N-terminal amino acid and electron microphotographs have been obtained that indicate a six-subunits structure (8). The evidence, though not conclusive, strongly suggests that the unit of weight 480,000 is an aggregate of some 6-8 identical, or quite similar, subunits. The aforementioned dissociating agents irreversibly inactivate the enzyme.

The present investigation was prompted by the finding of Blatter et al. that treatment of urease in pH 9 TRIS buffer with 9 parts (v/v) of 1,2-propanediol causes partial dissociation, to a product of M.W. about 240,000 that is catalytically active (9). We tested several other organic solvents and found that 1,2-ethanediol gave the best results, in that it acts rapidly, much more so than 1,2-propanediol at comparable concentrations, and that the enzymatic activity is completely preserved.

The dissociation of urease molecules into halves can also be realized by exposing the enzyme to 0.1M acetate buffer of pH 3.5. In these conditions, however, an irreversible denaturation also takes place, albeit more slowly than the dissociation, and lowers the enzymatic activity of the product (10). The dissociation of urease in similar conditions -- citrate-phosphate buffer of pH<4.9 containing 20-5% sucrose -- has also been reported by Tanis and Naylor (11).

EXPERIMENTAL

Phosphate-EDTA buffer, 0.02M, pH 7, contained 3.13 g of $Na_2HPO_4.7H_2O$, 1.15 g of $NaH_2PO_4.H_2O$ and 0.3723 g of EDTA, disodium salt, per liter; TRIS-EDTA buffer, pH 9, contained 10 g of tris(hydroxymethyl)aminomethane and 1 g of EDTA, acid form, per liter. Urease was prepared by the procedure of Mamiya and Gorin (12), with the following slight, but important, modifications: the solvent used to extract 100-g portions of jack-bean meal was prepared by diluting 160 ml of acetone to 500 ml with a solution 0.01M in 2-mercaptoeth-

anol and 0.001M in EDTA, pH 7; a solution containing 0.01M 2-mercaptoethanol and 0.001M EDTA was also used to dissolve the urease, prior to recrystallization with citrate-acetone. The preparations of crystalline urease were no more than 2 months old, and stock solutions were prepared fresh as needed. The concentration of enzyme was estimated using the factor $E_{1cm}^{0.1\%}$ 0.64 at 278 nm (13). The mixtures of buffer and ethanediol were made up carefully by weight, but the specification of composition refers to relative volumes; i.e., a 33% solution was mixed from 1 vol of glycol and 2 vols of buffer containing urease.

Electrophoresis was conducted in the multichannel vertical apparatus described by Blattler (14); the medium was 5% acrylamide gel-TRIS buffer. The gels were stained by immersing for at least 12 hr in a medium containing 50 mg of amido black, 500 ml of methanol, 500 ml of water, and 100 ml of glacial acetic acid; soaking in a mixture of the latter three substances was then used to remove excess dye. Ultracentrifugal analysis was done with a Beckman-Spinco ultracentrifuge, Model E, at 20°. Data for the viscosity and density of 1,2-ethanediol-water mixtures were taken from Timmermans (15).

RESULTS AND DISCUSSION

Urease in TRIS-EDTA gave, on ultracentrifugal analysis, a single peak, with a sedimentation coefficient s of 18 (Svedbergs). This is in agreement with previous reports (3,4), and corresponds to a weight of 480,000. Electrophoresis usually gave a single intense band and some faint additional bands; the components responsible for the latter bands amounted to <10% of the total. Treatment of urease with 10% glycol produced no appreciable change either in the ultracentrifugal or in the electrophoretic patterns, except, of course, that the s value was decreased; the decrease was only to the extent expected because of the increased viscosity and density of the medium.

On the other hand, treatment of the urease solution with 33-50% 1,2-ethanediol caused its conversion to a product which migrated faster on electro

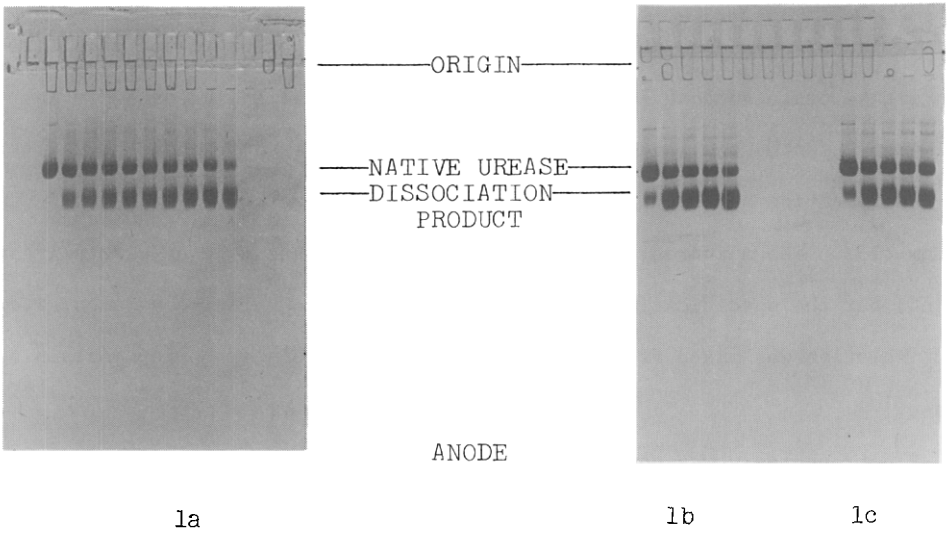

Fig. 1. Results of electrophoresis experiments, in the following conditions: medium, 5% acrylamide and TRIS buffer; gel dimensions, 13 x 15.3 x 0.4 cm; potential: 14 v/cm; duration, 2 hr. Mobility in 10^{-5} cm^2v^{-1} sec^{-1}: top band, native urease, 2.8; bottom band, dissociated urease, 3.8.
Fig. 1a (left). Samples containing about 25 mg/ml of urease in TRIS-EDTA treated with 50% glycol at 20° for following lengths of time (left to right): control; 4 min; 6 min; 8 min; 10 min; 12 min (approximately equal amounts in each band); 14 min; 18 min; 24 min; 36 min.
Fig. 1b (middle). Samples exposed to 33% glycol and TRIS-EDTA at 20°: control (10 min); 11 hr; 12 hr; 14 hr; 18 hr; 21 hr; 22 hr.
Fig. 1c (right). Samples exposed to 33% glycol and phosphate-EDTA at 20°; control (10 min); 11 hr; 12 hr; 14 hr; 18 hr; 21 hr; 22 hr.

phoresis and sedimented more slowly in the ultracentrifuge. In 33% glycol, the conversion took place over several hours, while in 50% glycol it occurred quite rapidly. In order to obtain more precise measurements of the rate, the following procedure was developed. The desired amount of glycol was added to the urease solution. Then, at precisely timed intervals, aliquots were taken and diluted with buffer to 10% glycol. In this medium no change in the electrophoretic or ultracentrifugal patterns took place in the time required to conduct the desired measurements.

Fig. 1 shows representative results, obtained by electrophoresis after treatment of urease in TRIS-EDTA with (1a) 50% glycol and (1b) 33% glycol. It may be seen that, qualitatively, the same change took place in both media;

a faster band gradually developed, at the expense of the slower, native urease band. The rates of the process depended very critically on the glycol concentration. The half-reaction periods, i.e. the times required to develop equal intensity in the two bands, was about 13 min in 50% glycol, and 13 hr in 33% glycol. A third determination, not shown, was done in 45% glycol; the half-reaction period in this case was 1.5 hr. The observation that no appreciable change would occur in 10% glycol is in keeping with the trend evidenced by these results.

Fig. 2 shows the results obtained when urease that had been exposed to 50% glycol for 12 min was diluted to 10% glycol and then subjected to ultracentrifugal analysis. It may be seen that nearly one half of the urease had

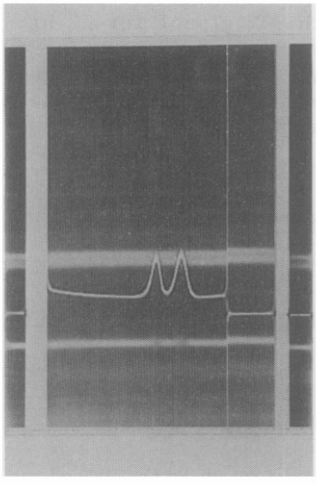

Fig. 2. Ultracentrifugal pattern of urease exposed to 50% glycol and TRIS buffer for 12 min, then diluted to 10% glycol. s/s' for peaks is 1.56.

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been converted to a product with a smaller s value, in accordance with the results obtained by gel electrophoresis. In 33% glycol, qualitatively the same results were obtained, but at a much slower rate, that also corresponded to the electrophoresis experiments.

When urease was dissolved in phosphate-EDTA, its behavior toward dissocia-

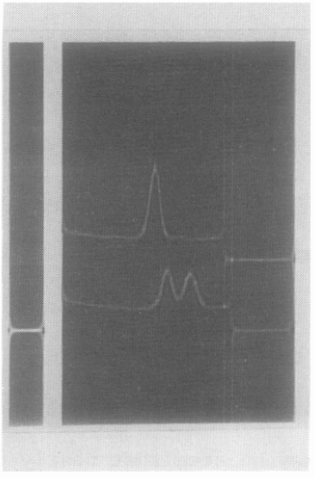

Fig. 3. Ultracentrifugal patterns given by urease that was dissolved in phosphate and treated with 33% glycol for 12 hr, then divided into two aliquots. Patterns are superimposed by using regular and wedge-window ultracentrifuge cells. Top pattern: aliquot diluted to 10% glycol with phosphate. Bottom pattern: aliquot diluted to 10% glycol with TRIS buffer; s/s' for peaks is 1.53.

tion by 33% or 50% glycol was quite similar to that in TRIS buffer. This may be seen, for the case of 33% glycol, by comparing Figs. 1b and 1c. An important difference was noted, however, when the solution was diluted to 10% glycol, or less, with pH 7 buffer. In this event, reassociation occurred; this is illustrated by Fig. 3.

Fig. 4 shows that the treatment with 50% glycol caused no appreciable change in the activity over a period of 200 min. This is much longer than the time necessary for the dissociation to take place.

The fact that the activity is quantitatively retained indicates that treatment with 1,2-ethanediol does not alter the internal conformation of the subunits. The ratio of the s values for native urease and for its dissociation product is 1.54 (cf. Figs. 2 and 3). This corresponds very closely to the change expected if the molecular weight were decreased to half, with no change in the frictional ratio, i.e. $s/s' = 2^{2/3} = 1.586$. As we have seen, there is good

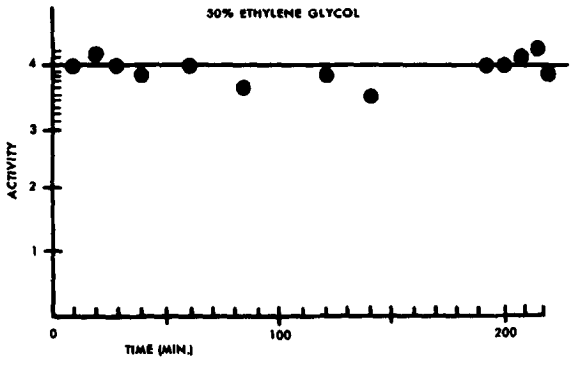

Fig. 4. Activity vs. time, urease in 50% glycol-TRIS buffer.

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evidence for believing that the unit of weight 480,000 is an aggregate of 6-8 subunits. If these subunits are themselves symmetrical and close-packed, the division into halves can indeed take place without much change in the asymmetry.

What causes the dissociation? The relationship between it and the pH suggests that the charge in the molecule is a factor. But, at pH 7 and 9, charge alone is not enough. The addition of organic solvent would favor dissociation by increasing the repulsive force and reducing the hydrophobic interaction that tends to exclude certain parts of the molecule from bulk water. From this argument we should, however, expect that 1,2-propanediol be more effective than ethanediol. A possible explanation of the results is that a nice balance between hydrophilic and hydrophobic character is necessary for the solvent molecules to "make their way" into the developing crevice between the two parts.

The fact that reassociation takes place in 10% glycol and pH 7 but not at pH 9 can be rationalized in two ways, that cannot be distinguished by the present evidence. At the higher pH both urease and its dissociation product bear a higher electrostic charge. It may be that urease in pH 9 TRIS buffer is unstable with respect to dissociation even in 10% glycol, but that the rate

of the process is so slow it could not be observed in our experiments. Alternatively, it may be that urease is stable relative to its dissociation product, but that the rate of recombination is too slow to measure.

In view of the results described we believe it should be interesting to test the dissociating ability of 1,2-ethanediol and congeners on other multichain enzymes.

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