# Production of the Urease Half-Unit at Alkaline pH[1] (36490)

William N. Fishbein and K. Nagarajan

*Biochemistry Branch, Armed Forces Institute of Pathology, Washington, D.C. 20305*

The traditional form of urease normally isolated from jack bean meal, α-urease (mol wt 480,000) (1), is cleaved to catalytically active half-units, $A_1$-urease (mol wt 240,000), by treatment with glycol (2, 3), titration to pH 3.5–4.5 (4, 5), and at very high ionic strengths (6). The latter two observations suggest that the dissociation is accomplished by charge separation; if so, one might expect the same phenomenon to be produced by alkalinization, but this has not been reported. We have demonstrated the production of small amounts of $A_1$-urease from α-urease by titration to pH 3.5–4.5, but did not observe the appearance of $A_1$-urease after alkaline titration to pH values as high as 11.3, where the enzyme is inactivated (5). However that study was performed using a catalytic stain for urease activity rather than a protein stain. It occurred to us that charge separation might result in an inactive $A_1$-urease at alkaline pH for reasons trivial to the overall structure; that is, specific chemical or conformational changes might inactivate the substrate site without influencing the overall aggregate state. That this is in fact the case is shown below.

*Experimental procedure.* Pure α-urease (130–150 Sumner units/mg) was prepared from jack bean meal as previously described (5, 7). Aliquots of a solution containing 0.5 mg urease/ml, 5 m*M* Tris–HCl–0.1 m*M* EDTA (pH 7.4) were titrated with μl additions of *N* NaOH using a Beckman research pH meter equipped with a sodium ion insensitive combination microelectrode. After 1 hr standing, the samples were applied, equilibrated, and electrophoresed in 6% acrylamide gels in 0.05 *M* Tris–acetate–1 m*M* EDTA, pH 8.5 (3, 7). One gel was used for Coomassie blue staining and densitometry (8), the other for catalytic staining for urease activity (9).

*Results.* Figure 1 displays the pertinent information, provided by the Coomassie blue stained gel. Slot 1 shows the location of the active α-urease, while slot 2 shows the location of the well-categorized $A_1$-urease produced by treatment with 90% glycol for 1 hr (7, 10). The remaining slots contain samples titrated to the pH noted, and stored for 1 hr at 25°. All samples equilibrated to pH 8.5 before electrophoresis, as is evident from the constant position of α-urease. The latter also shows that inactivation, which occurs at pH 11.3 (5), is not synchronous with denaturation, and does not alter α-urease mobility. Slots 6 and 7 show gradually increasing amounts of $A_1$-urease formed as the pH is elevated to 11.6–11.9. Densitometric evaluation of slot 7 indicated 8% weight conversion to $A_1$-urease, quite similar to the amount produced by acid titration (5). At 0.5 pH unit higher (slot 8) the enzyme has been fully denatured, and appears as a polydisperse stain. The catalytic stain showed no active forms at pH > 11.2, and progressive decrease in the amount of active α-urease from pH 10–11.2, as in previous studies (5).

*Discussion.* Except for the absence of activity, the effects of acid and alkaline titration now appear in good agreement. In each

---

[1] This work was supported in part by a research grant, Project 3A061102B71R, from the Medical Research and Development Command, U.S. Army, Washington, DC 20012, and in part by Public Health Service Research Grant AM 10960 from NIAMD, Department of Health, Education and Welfare, under the auspices of Universities for Research and Education in Pathology, Inc. The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

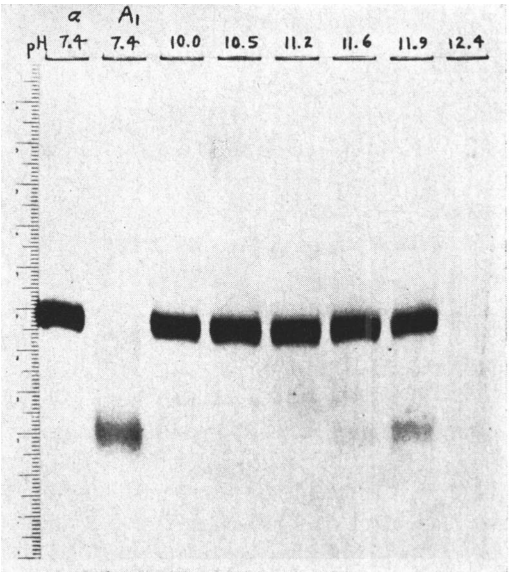

FIG. 1. Acrylamide gel electrophoresis of urease after alkaline titration. The first two slots show the location of standard preparations of α-urease and $A_1$-urease (see text). The remainder show samples of α-urease titrated to the pH noted above each slot for 1 hr prior to electrophoresis. The appearance of $A_1$-urease at pH 11.9 occurs just below the level (pH 12.4) producing full denaturation with loss of all homogeneity. Conditions: 6% gel with 5% cross linker, 6 hr run at 300 V in 0.05 *M* Tris–acetate–1 m*M* EDTA, pH 8.5.

case a small fraction of the α-urease that is dissociated to the $A_1$ form, survives return of the pH to neutral. In each case the effect is produced at a pH 3.5–4.5 units away from neutral, and within 0.5–1 pH unit of the point where full denaturation occurs, as indicated by inactivation *and* electrophoretic polydispersity. The small amount of $A_1$-urease present may reflect reassociation during equilibration of the pH to 8.5. At pH 12 the dissociation may be complete, as is the case upon acid titration (4).

The results are most simply explained by charge separation consequent to charge conversion. Upon acid titration, anionic groups are protonated until primarily cationic charges remain, resulting in ionic repulsion generating the half-units. The reverse situation occurs at alkaline pH, but the net effect is the same, the charge repulsion now being due to the excess of anionic charges. In each case, slight further charge conversion is sufficient to produce enough charge repulsion to fully denature the enzyme. The dissocation of α-urease to $A_1$-urease at high ionic strengths in 4 *M* citrate (pH 6) (6) is similarly explicable as a case of charge separation, in this case due to charge competition. At very high ionic strengths the opposite charges of the $A_1$ units lose their preferential attraction for one another in the face of a vast excess of small ions. In effect, the $A_1$-urease units are salted apart. This interpretation implies that the predominant bonding forces between $A_1$-urease units are electrostatic rather than hydrophobic as has been suggested by others (10). However, alternate interpretations of the action of citrate, which may involve specific ligand effects (3) or nonpolar interactions, prevent any certain conclusion.

*Summary.* α-Urease (mol wt 480,000) inactivated by titration to pH 11.3 has the same electrophoretic mobility as active α-urease. Titration to pH 11.6–11.9 results in partial dissociation to $A_1$-urease (mol wt 240, 000) as judged by electrophoretic mobility equivalent to that of active $A_1$-urease produced by glycol. At slightly higher pH (12.4), full denaturation of the enzyme occurs. The results are similar, quantitatively, to those produced by acid titration, and qualitatively to the dissociation produced by high ionic strengths near neutral pH. Charge separation is thus indicated as the mechanism.

---

1. Sumner, J. B., Gralen, N., and Eriksson-Quensel, I.-B., J. Biol. Chem. **125**, 37 (1938).
2. Blattler, D. P., Contaxis, C. C., and Reithel, F. J., Nature (London) **216**, 274 (1967).
3. Fishbein, W. N., Ann. N.Y. Acad. Sci. **147**, 857 (1969).
4. Tanis, R. J., and Naylor, A. W., Biochem. J. **108**, 771 (1968).
5. Fishbein, W. N., and Nagarajan, K., Arch Biochem. Biophys. **144**, 700 (1971).
6. Lynn, K. R., Can. J. Biochem. **48**, 632 (1970).
7. Fishbein, W. N., Nagarajan, K., and Scurzi, W., J. Biol. Chem. **245**, 5985 (1970).
8. Fishbein, W. N., Anal. Biochem. **46**, 388 (1972).
9. Fishbein, W. N., "Fifth International Symposium on Chromatography and Electrophoresis," p. 238. Ann Arbor-Humphrey Science Press, Ann Arbor, MI (1969).
10. Contaxis, C. C., and Reithel, F. J., J. Biol. Chem. **246**, 677 (1971).

---

Received Jan. 21, 1972. P.S.E.B.M., 1972, Vol. 140.