# A LOW MOLECULAR-WEIGHT FORM OF UREASE

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Sedimentation and enzymatic-activity studies of urease so far have revealed the presence of forms with average $S_{20,w}$ values of 19, 28, 36 and 46 (Sumner, et al., 1938; Kuff, et al., 1955; Creeth and Nichol, 1960). These components have been shown not to be affected by dilution or standing, thus indicating that they are not in rapid equilibrium (Creeth and Nichol, 1960). Recently, Siegel and Monty (1965) fractionated Sigma type C-1 urease on columns of Sephadex G-200. Their results showed that species of active urease having Stokes radii of 61, 79 and 92 angstroms correspond to the $S_{20,w}$ values (obtained from Creeth and Nichol, 1960) of 19, 28 and 36.

In some experiments done at low ionic strengths Creeth and Nichol (1960) report the occurrence of a possible 12S component of urease. Working with urease samples of different percentage purities on the analytical ultracentrifuge Sehgal (1964); Sehgal and Naylor (1964) concluded that urease may have a 12S form. In the present paper we have shown that in the different samples analyzed the 12S peak is that of active urease.

## MATERIALS AND METHODS

Sigma type V urease (lot number 113B-7860) and defatted Sigma jack bean meal were obtained from the Sigma Chemical Company. Our untreated meal was prepared by processing dry mature jack bean seeds, with seed coats removed, through a stainless steel pulverizing mill. Watermelon and jack bean seeds were soaked in water 24 hours prior to extraction.

Each enzyme source was extracted with 0.05M phosphate buffer, pH 7.0 containing 0.1% mercaptoethanol. Each extract was clarified by centrifuging at 30,000 x g for 20 minutes followed by centrifuging at 110,000 x g for 30 minutes.

All sedimentation coefficient values were obtained using the technique of sucrose density gradient centrifugation (Martin and Ames, 1960). Linear 20 to 5 per cent gradients containing 0.02M phosphate buffer, pH 7.0 plus 0.1% mercaptoethanol were used. The sample containing 0.15 ml of extract and 0.05 ml of catalase, used as a sedimentation standard, was layered on top of the 4.6 ml gradient and centrifuged at 4°C for 9.08 hours at 32,800 rpm using the SW-39 rotor in the Spinco Model L preparative ultracentrifuge. During the run the temperature inside the swinging bucket was held to ±0.4°C (measured with a thermometer before and after centrifugation in a bucket containing only a sucrose gradient). Forty-four fractions were collected using a fractionating device that punctured the bottom of the tube.

The enzymatic assays were carried out immediately at room temperature.

Urease was assayed in microconway dishes (Conway, 1962) and the ammonia evolved was titrated with acid. This assay is a modification of Sumner's procedure (Sehgal, 1964). The reaction time was 30 minutes for the 12S and 26S peaks and 5 minutes for the 18S region.

## RESULTS AND DISCUSSION

The data presented in Table I show the absence of a 12S

TABLE I

FORMS OF UREASE AND THEIR $S_{20,w}$ VALUES[1]

| MATERIAL | PEAK A | PEAK B | PEAK C |
|---|---|---|---|
| Soaked Watermelon Seeds | - | 18.6 | - |
| Soaked Jack Bean Seeds | 12.0 | 18.5 | - |
| Untreated Jack Bean Meal | 12.8 | 19.6 | - |
| Sigma Defatted Jack Bean Meal | 12.7 | 19.0 | 26.5 |
| Sigma Type V Urease[2] | 12.7 | 18.7 | 25.4 |

[1]Based on a $S_{20,w}$ value of 11.3 for catalase.

[2]Essentially a dialyzed jack bean extract (personal communication from Sigma Chemical Company).

component only in watermelon seeds. In most of the materials, the amount of 12S and 26S components together comprised about 3 per cent of the total urease units. In Sigma type V urease we found that the amount of 12S component alone was

about 4 per cent and the 26S peak comprised 1.4 per cent of the total urease units. With the techniques employed in the present study, the materials investigated did not show the presence of 36S or higher forms of urease.

Siegel and Monty (1965) using their values of Stokes radii and $S_{20,w}$ values obtained from the literature calculated the approximate molecular weights of different species of urease. Assuming a molecular weight of 483,000 for the 19S peak they concluded that the 28S peak was a dimer and that the 36S peak corresponds to a trimer. However, if the 12S peak obtained by us is assumed to be a monomer then the 19S form represents a dimer*. The 26S peak shown in Table I probably corresponds to the 28S peak reported in the literature and may represent either a trimeric or a tetrameric form of urease.

In summary, we have shown that the 12S peak obtained by means of sucrose density gradient centrifugation is that of active urease and may possibly represent the monomeric form of the enzyme. Further work on the isolation and characterization of the 12S component is in progress.

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*Calculated by applying the relationship: $\frac{S_1}{S_2} = \left(\frac{MW_1}{MW_2}\right)^{2/3}$

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