LXVI. IS THERE A CO-ENZYME FOR UREASE?

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WHEN it was discovered in this laboratory [Sumner, 1926, 1, 2] that jack bean urease could be separated as globulin crystals and that these could be recrystallised without loss of activity we naturally believed this evidence sufficient to prove that no co-enzyme for urease could exist. For it was to be expected that crystallisation would separate the urease from its co-enzyme, if such were present in the jack bean, and that crystalline urease would be partly inactive and would become still less active on recrystallising. On the contrary, recrystallisation from both 32 % acetone and from 30 % alcohol has been found to cause an increase in activity instead of a decrease. Moreover, it was noted at the outset that the decrease of enzyme activity that occurs when the crystals separate from a 32 % acetone jack bean extract is almost exactly equal to the activity of the crystals themselves, and a statement was made at that time that no co-enzyme for urease could be present in the jack bean [Sumner, 1926, 1]. Nevertheless, co-urease has been discussed in a recent monograph on enzymes without any mention being made of the practical certainty that there is no such substance [Haldane, 1930]. We have therefore considered it necessary to repeat some of the experiments of Onodera [1915] and Kato [1923], who are responsible for the claim for the existence of co-urease. It might be argued that our findings apply only to jack bean urease and that they are not valid for soya bean urease, which was the enzyme used by the authors mentioned above. However, we have shown recently that soya urease is inhibited by highly purified jack bean anti-urease and that it gives the precipitin test with this anti-urease also, which establishes an immunological relationship between the two enzymes [Kirk and Sumner, 1932].

Onodera [1915] brought forward evidence for the existence of a co-enzyme for soya urease, although he states: "Indeed its nature, as will be seen, throws some doubt on its claim to the title." He prepared soya urease according to the method of Van Slyke and Cullen [1914]. Urease dialysed for 5 days against distilled water lost nearly all its activity, but on adding to this some fresh urease the lost activity was partly restored. No co-urease could be found in the dialysate. Urease treated with acid, or alkali, or heated even to boiling lost its co-enzyme, while the urease proper was not destroyed, as could be deduced from the fact that addition of fresh urease partly restored the activity.

As the result of experiments which need not be discussed here Onodera further claimed that the co-enzyme consisted of two substances, one dialysable and the other not dialysable.

Kato [1923] stated that soya urease preparations contain two essential constituents, the enzyme proper and an unknown substance *X*. He believed substance *X* to react with urea to form a compound which could be hydrolysed by urease. He stated that urea could not be hydrolysed in the absence of substance *X* and that urease proper was inactivated by urea if *X* were absent. Kato used phosphate buffer in certain experiments for determining urease activity and in other experiments he used no buffer. He claimed that it was impossible to compare the activities of two different urease preparations and showed (his Table I) how various quantities of preparation A and of preparation B gave values for urease content that fluctuated in a most extraordinary manner. Here no buffer was employed.

By treating soya urease with freshly precipitated calcium phosphate he obtained two fractions, a filtrate and a precipitate. The filtrate alone had a weak urease activity and this was not increased by adding glycine. The precipitate had by itself a weak urease action and this was increased by adding either some glycine or some of the filtrate. In determining the urease action he used no buffer (his Table 36).

Lövgren [1921] has repeated some of Onodera's experiments, using phosphate buffer, and has concluded that Onodera's claim for co-urease is unconvincing and that the results are to be explained as due principally to a p_H effect.

Our first explanation of the results of Onodera and Kato was the same as Lövgren's. For one could expect that urease *plus* inactivated urease would decompose urea more rapidly than urease alone, since the inactivated urease would serve as buffer to some extent and would maintain a more favourable p_H . However, we have tested this out experimentally and have found that while urease *plus* inactivated urease always hydrolyses more urea than does urease alone, the difference is slight. Table I illustrates this.

Table I.

	Ammonia-N (mg.)
5 cc. Van Slyke soya urease heated 10 mins. in boiling water. Added 45 cc. redistilled water. Added 1 cc. 0.5 % urease. Brought to 30° in bath. Added 10 cc. 3 % urea. After 4 hours added 25 cc. <i>N</i> HCl. Analysed for ammonia by aeration	16.5
To 1 cc. 0.5 % soya urease added 50 cc. redistilled water. Brought to 30° in bath. Added 10 cc. 3 % urea. After 4 hours added 25 cc. <i>N</i> HCl	15.4

The buffering power of soya urease solution is small and the results of Onodera and Kato cannot be accounted for as due solely to p_H effect. However, there is a more plausible reason for their findings. This is the protective effect of the colloids present in inactivated urease against traces of heavy metals in distilled water. The experiments in Table II show how great a difference is

made when a minute amount of copper sulphate is added on the one hand to soya urease *plus* boiled urease and, on the other hand, to soya urease containing no boiled urease.

Table II.

	Ammonia-N (mg.)
5 cc. 5 % Van Slyke soya urease heated 10 mins. in boiling water. Added 44 cc. redistilled water. Added 1 cc. 0.5 % urease and 1 cc. containing 0.035 mg. CuSO_4 . Brought to 30° (15 mins.). Added 10 cc. 3 % urea. After 4 hours added 25 cc. <i>N</i> HCl	16.0
To 1 cc. 0.5 % urease added 49 cc. redistilled water and 1 cc. 0.035 mg. CuSO_4 . Brought to 30° (15 mins.). Added 10 cc. 3 % urea. After 4 hours added 25 cc. <i>N</i> HCl	0.13

In Table III the same procedure was followed except that 5 cc. of 9.6 % neutral phosphate were added in place of 5 cc. of the water.

Table III.

	Ammonia-N (mg.)
5 cc. 5 % Van Slyke soya urease heated 10 mins. in boiling water. Added 39 cc. redistilled water. Added 1 cc. 0.5 % urease, 1 cc. containing 0.035 mg. CuSO_4 and 5 cc. phosphate. Brought to 30° (15 mins.). Added 10 cc. 3 % urea. After 4 hours added 25 cc. <i>N</i> HCl	30.1
To 1 cc. 0.5 % urease added 44 cc. redistilled water, 1 cc. containing 0.035 mg. CuSO_4 and 5 cc. phosphate. Brought to 30° (15 mins.). Added 10 cc. 3 % urea. After 4 hours added 25 cc. <i>N</i> HCl	0.86

It is of course impossible to be certain that the distilled water employed by Onodera and Kato contained traces of heavy metal, but it is very probable that it did. We have carried out experiments both with crystalline urease and with impure soya bean urease in which the water employed was, as usual, redistilled from glass. Our analyses, as will be seen below, show that when dialysed urease is added to fresh urease the mixture possesses exactly the sum of the activity of the two components.

All the glassware was free from adsorbed films of heavy metal. Our reagents were carefully purified to assure freedom from heavy metals. The method for estimating urease activity was the one employed in this laboratory for the past 4 years [Sumner and Hand, 1928]. Crystalline urease was prepared by our usual method. Soya urease was prepared by extracting 1 part of defatted, dust-fine meal with 5 parts of water for 1 hour, followed by centrifuging. The soya beans were first ground coarsely in a coffee mill equipped with steel burrs, then separated from the hulls by an air blast and extracted with light petroleum. After evaporating off the solvent the material was ground in a porcelain ball mill and sifted through silk bolting cloth.

Table IV.

Crystalline jack bean urease 1 cc. (1140 units) was dialysed under toluene in a collodion bag against 4 changes of water (450 cc. each) in an ice-chest for 72 hours. The material was washed with a little neutral phosphate and diluted to 50 cc. The solution was turbid, showing denaturation. 10 cc. were diluted to 92 cc. and this solution was tested for activity both alone and after mixing with an equal vol. of fresh diluted urease.

	Urease units
1 cc. dialysed diluted urease	0.93
1 cc. fresh diluted urease ...	1.07
1 cc. mixture 1:1	0.98

Calculation shows that during dialysis the urease lost 62 % of its activity. If the activity of the dialysed urease had been completely restored by addition of fresh urease the mixture should have contained 1.75 units per cc. However, it had an activity of only 0.98 unit, while the average of the two components gives 1.00.

Table V.

5.0 cc. of soya urease were dialysed under toluene in the ice-chest 15 days. The water (450 cc.) was changed every day. During this time the soya urease lost 55 % of its activity. Presumably the reason why the loss was not greater was because the water was free from heavy metals. The material was washed out with neutral phosphate and diluted to 42 cc. 5 cc. of the original urease were diluted to 42 cc. and the 2 solutions were tested for urease activity both separately and after mixing 1:1.

	Urease units	
1 cc. diluted dialysed urease	0.21,	0.23
1 cc. fresh diluted urease ...	0.49,	0.48
1 cc. mixture 1:1 ...	0.36,	0.36

Here again the activity of the mixture, 0.36 unit, is almost exactly equal to the sum of the activities of the two components, 0.35 unit. If any co-urease were present this would not be true.

CONCLUSION.

Active urease *plus* inactivated urease produces only slightly more ammonia from urea than active urease alone.

If traces of heavy metal are present urease is inactivated less readily in the presence of boiled urease than in its absence.

The results of Onodera [1915] and Kato [1923] cannot be accepted as an indication of the presence of a co-urease in their preparations, but are probably to be explained as due to a protection of urease against heavy metal impurities.

Our experiments with crystalline urease and soya urease show that there is no evidence of a co-enzyme for urease.

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