On the mechanism of urease action

In the urease-catalyzed hydrolysis of urea, the formation of ammonium carbamate has been demonstrated by many workers[1], and in a particularly clear and simple manner by the experiments of SUMNER, HAND AND HOLLOWAY[2]. These experiments, however, did not establish unequivocally whether (a) carbamate is the intermediate in the formation of NH_3 and CO_2, or (b) NH_3 and CO_2 are produced first, and combine to give the carbamate. In the original discussion of their experiments, SUMNER *et al.*[2] inclined toward the former view, but a more recent statement of SUMNER[3] seems to favor the latter. Experiments designed to answer the question unequivocally were performed only recently by WANG AND TARR[4], who studied the hydrolysis reaction in ^{18}O-labelled water, and concluded that, in citrate buffer of pH 5.5, carbamic acid was the intermediate in the formation of CO_2. The following experiments indicate that carbamate is also the intermediate when no buffering agents are added and the pH, spontaneously established by the products, is about 9[5].

In unbuffered solutions, the products are mainly NH_4^+ and HCO_3^- ions, as well as carbamate, in equilibrium with one another. The concentration of carbamate present at equilibrium naturally decreases with increasing dilution of the aqueous solution, and at a formal concentration of 0.02 *M* nitrogen, practically no carbamate is present[6]. However, since the reaction of CO_2 with NH_3 is relatively fast compared to the hydration of CO_2 to HCO_3^- at pH 9, when aqueous solutions of NH_3 and CO_2 are mixed carbamate first forms[7] and then hydrolyzes.

This can be demonstrated by utilizing the facts that carbamate does not give a color with Nessler's reagent[2], and that carbamate hydrolyzes instantaneously and completely in 1 *M* HCl, but only quite slowly in 0.01 *M* NaOH[8]. In Expt. 1, stoichiometrically equivalent amounts of NH_3 and CO_2 in aqueous solution were mixed at 0°, at a formal NH_3 concentration approximately 0.02 *M*; after exactly 2 min two 1.00-ml aliquots were withdrawn and added to excess 0.01 *M* NaOH and to 1.00 ml 1 *M* HCl, respectively, and Nesslerized soon afterwards. The absorbancies developed measure the concentration of NH_3, and from the difference the concentration of carbamate in the original solution could be calculated.

In Expt. 2, urea was hydrolyzed in the presence of urease at 0°, and, after 2 min, aliquots of the reaction mixture were treated with HCl and NaOH and then Nesslerized, as in Expt. 1; the strong acid and base stop the enzyme action, as well as interacting with carbamate in the manner already discussed. Although the final concentration of NH_3 in the reaction mixture after acid hydrolysis was about the same as that taken initially for Expt. 1, the amount of urease having been deliberately adjusted so that this would be the case, the concentrations of NH_3 and CO_2 were zero at the beginning of this experiment, and lower than in Expt. 1 throughout the reaction period. Despite this, it is seen that the amount of carbamate formed in Expt. 2 was greater. Since the rate of formation of carbamate depends on the concentration of NH_3 and CO_2[7], it is clear that the carbamate could not have been derived from reaction between these two substances. Expt. 2 was done with once-recrystallized urease, prepared according to SUMNER[8], which had an activity of about 10,000 units/g (Sample I); Expt. 3 was done with a more impure but crystalline preparation (Sample II). The percentage of carbamate is somewhat lower in this case, but still greater than that which might have formed under the conditions by combination of NH_3 and

CO_2. When a still less active, non-crystalline preparation of urease was used (Sigma Chemical Co., Saint Louis, "Type V", activity about 1,500 units/g) a much lower proportion of carbamate was found, and the significance of the result was ambiguous; apparently, impure samples of urease may be associated with substances that catalyze the hydrolysis of carbamate, and only purified preparations give significant results.

A more striking demonstration of the mechanism of urease action can be made with the aid of carbonic anhydrase. KREBS AND ROUGHTON[9] first utilized this enzyme in an attempt to study the problem, but the design of their experiments did not permit them to distinguish between the two possibilities already discussed. However, the experiments described below attest to the soundness of their suggestion.

Expt. 4 was conducted with the same materials and in the same manner as Expt. 1, except that 250 μg of 0.1 % carbonic anhydrase (Worthington Biochemical Corp., Freehold, N.J.) was added to the NH_3 solution just before mixing it with CO_2; it is seen that no appreciable amount of carbamate was formed. This is in accordance with what has been said concerning the position of equilibrium and the relative rates of reaction of CO_2 with NH_3 and H_2O, respectively; carbonic anhydrase, of course, strongly catalyzes the latter reaction.

Expt. 5 was conducted like Expt. 2, except that carbonic anhydrase was added. It is seen that a considerable amount of carbamate was formed, although Expt. 4 shows that no appreciable combination of NH_3 and CO_2 could occur under these conditions; it is clear that the carbamate must have been formed directly from urea. The same amount of enzyme was used in Expts. 5 and 2, and it is seen that considerably less hydrolysis of urea took place in the presence of anhydrase; this is likely due to inhibition of urease by impurities in the anhydrase preparation. Expt. 6 was conducted like Expt. 3, with urease Sample II, but the amount of enzyme taken was increased to offset the inhibitory action of anhydrase, and produce comparable amounts of NH_3 after acidification. The anhydrase hastens the hydrolysis of carbamate, as might be expected from the mechanism of the reaction[6], so that less is found in the presence of anhydrase; but the significant fact is that *any* is found under these conditions.

In the performance of these experiments much trouble was caused by turbidity in the Nesslerized solutions; this is probably due in part to the unavoidable presence of CO_2. Clear solutions and reproducible results were obtained as follows. The stock solution was prepared by mixing 30 g KI, 22 g I_2, 28 g Hg, and 20 ml water until the mixture was yellowish green, diluting to 400 ml, and decanting from excess Hg[8].

TABLE I

CARBAMATE CONTENT OF REACTION MIXTURES

Expt.	*Reaction mixture*	*Absorbance after Nesslerization*		*% Carbamate*
		Direct	*After acid hydrolysis*	
1	0.24 *M* NH_3 + 0.12 *M* CO_2	0.176	0.261	32
2	3 % Urea + Urease I	0.137	0.244	44
3	3 % Urea + Urease II	0.143	0.237	39
4	As in 1 + Anhydrase	0.252	0.254	~0
5	As in 2 + Anhydrase	0.140	0.182	23
6	As in 3 + Anhydrase	0.194	0.245	21

The reagent solution was mixed from 75 ml stock solution, 125 ml 5.0 *M* NaOH, and 300 ml water. The sample was added to about 80 ml 0.01 *M* NaOH, 10 ml reagent solution was added with vigorous shaking, and the mixture diluted to 100 ml with 0.01 *M* NaOH. Absorbancies were determined in 1-cm cells with a Beckman DU spectrophotometer at 480 mμ.

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Uridine diphosphoglucose in banana fruit

UDPG is an intermediate in the synthesis of sucrose by enzyme systems extracted from higher plants[1-3] and has been identified in seedlings of mung bean (*Phaseolus aureus* Roxb.)[4] and in leaves of sugar beet (*Beta vulgaris* L.)[5]. The isolation of this compound from banana fruit, in which marked synthesis of sucrose occurs after harvest[6,7], is reported in this communication.

Banana fruit (*Musa Cavendishii* L.) were obtained from wholesale fruit merchants before commercial ripening treatment. 600 g of peeled fruit tissue was extracted with ethanol by blending (1 ml ethanol/g fresh wt.). The slurry was filtered at 1° and adjusted to pH 7 with ammonia. This was filtered again, and the filtrate passed through a column (1 × 15 cm) of Dowex-1 resin (Cl form). Nucleotides were then displaced from the column with 0.1 *N* HCl. This solution was neutralized with ammonia and nucleotides adsorbed on charcoal (British Drug Houses Ltd.) previously activated by boiling. The charcoal was then eluted with 50% ethanol, which was

Abbreviation: UDPG, uridine diphosphoglucose.