ADDENDUM

The Amino Acid Composition of Jack-Bean Urease

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Analyses were carried out on twice-recrystallized freeze-dried urease (Bailey & Boulter, 1969) that had previously been dialysed for 24 hr. in the cold against running tap water, and then for 24 hr. against three changes of deionized water. Acid hydrolysis of urease (5 mg.) was carried out at 110° in evacuated heavy-walled Pyrex tubes with three-times-redistilled 5.7M-hydrochloric acid at a concentration of 0.2 ml. of acid/mg. of protein. The hydrolysis procedure was otherwise similar to that described by Moore & Stein (1963). The hydrolysate was applied to the column in 0.1M-hydrochloric acid.

Duplicate hydrolysates were analysed on a Technicon AutoAnalyzer with the 18 hr. elution system.

Determination of half-cystine. Total half-cystine was determined: (1) by adjusting the pH of the hydrolysed sample to approx. 6.8 and allowing it to stand exposed to air for 4 hr. to allow oxidation of any cysteine to cystine, followed by amino acid analysis; (2) as cysteic acid after performic acid oxidation at 2° by the method of Hirs (1956); (3) as *S*-carboxymethylcysteine in hydrolysates of CM-urease; (4) as *S*-aminoethylcysteine in hydrolysates of AE-urease.

Determination of tryptophan. Tryptophan was determined: (1) spectrophotometrically by the method of Goodwin & Morton (1946), with the modification due to Danielsson (1949) (this determination was carried out on twice-recrystallized urease that had not been dialysed; this was necessary since dialysis precipitated urease in a form difficult to redissolve); (2) colorimetrically by the method (procedure E) of Spies & Chambers (1948); (3) by titration with *N*-bromosuccinimide (Bailey & Boulter, 1969) by using the assumptions of Patchornik, Lawson & Witkop (1958).

Determination of amides. Amide was determined from the ammonia produced from urease by 1M-hydrochloric acid in 2 hr. at 110°. The ammonia was determined by the micro-diffusion method of Conway (1947) and the phenate-hydrochlorite method of Riley & Sinhaseni (1957).

Samples of twice-recrystallized urease were hydrolysed for 20, 70, 90 and 100 hr. The results shown in Table 1 are the averages obtained from 15 analyses each in duplicate. Recoveries of serine and threonine decreased with longer hydrolysis times and were

Table 1. *Amino acid recoveries from urease after acid hydrolysis*

The 20 hr. values are means $\pm$ S.D. of 15 determinations.

	Recovery (g. of amino acid residue/100 g. of dry protein)				
	Hydrolysis time (hr.)				Best value
	20	70	90	100	
Asp	11.14 $\pm$ 0.5	11.19	10.99	11.01	11.08
Thr	5.84	5.37	5.28	5.17	6.09*
Ser	4.19	3.44	3.03	2.96	4.53*
Glu	10.78 $\pm$ 0.5	10.63	10.86	10.61	10.72
Pro	5.29 $\pm$ 0.3	5.32	5.34	5.30	5.31
Gly	4.83 $\pm$ 0.2	4.84	4.99	4.74	4.85
Ala	5.75 $\pm$ 0.3	5.77	5.90	5.72	5.78
Val	5.49	5.84	5.89	5.85	5.86*
Met†	3.14 $\pm$ 0.3	3.22	3.32	3.25	3.23
Ile	6.81	7.66	7.65	7.60	7.64†
Leu	8.53 $\pm$ 0.2	8.56	8.60	8.60	8.57
Tyr	3.56 $\pm$ 0.2	3.55	3.51	3.40	3.56*
Phe	3.79 $\pm$ 0.2	3.82	3.83	3.81	3.81
Lys	6.77 $\pm$ 0.6	6.67	6.70	6.69	6.71
His	3.70 $\pm$ 0.3	3.71	3.75	3.69	3.71
Arg	6.41 $\pm$ 0.4	6.50	6.52	6.48	6.48

* Calculated from 20 hr. and 70 hr. values assuming first-order kinetics (Moore & Stein, 1963) for the destruction.

† Calculated from maximal recovery.

‡ Includes methionine sulfoxides.

recalculated by assuming that their breakdown followed first-order kinetics (Moore & Stein, 1963). Isoleucine and valine required a 70 hr. hydrolysis period for maximum recoveries.

The corrected amino acid composition of urease (Table 4) was derived from the data of Tables 1, 2 and 3.

Analyses of CM-urease and AE-urease showed complete conversion of half-cystine with the concomitant appearance of *S*-carboxymethylcysteine and *S*-aminoethylcysteine respectively, and no changes in the proportions of any other amino acids were detected (Table 2). Performic acid-oxidized

Table 2. *Determination of total half-cystine*

Method	Half-cystine (g. of amino acid residue/100g. of dry protein)
Acid hydrolysates, reoxidized by air at pH 6.8	1.42*
S-Carboxymethylcysteine in CM-urease, measured after acid hydrolysis	1.47
Cysteic acid, measured after performic acid oxidation and acid hydrolysis.	1.46†
S-Aminoethylcysteine in AE-urease, measured after acid hydrolysis	1.63‡

* Maximum value obtained from 20hr., 70hr., 90hr. and 100hr. hydrolysis.

† Corrected for 79% recovery after oxidation, since added cysteic acid broke down to the extent of 21% under these conditions.

‡ Based on a colour factor of 0.91 relative to lysine (Dopheide, Moore & Stein, 1967).

Table 3. *Determination of tryptophan*

See the text for details of the methods.

Method	Tryptophan (g. of amino acid residue/100g. of dry protein)
Spectrophotometric	1.27
Colorimetric	1.27
N-Bromosuccinimide	1.18

urease hydrolysates contained cysteic acid and no cysteine (Table 2), but about 35% of the total tyrosine was lost, probably because of conversion into chlorotyrosine (Thompson, 1954).

An amino acid analysis of urease has been published by Reithel & Robbins (1967). Comparison of the two analyses is hindered by the difference in total nitrogen recovery. However, on the less strenuous comparison of molar ratios, agreement is not good. Differences less than 10% are only found for seven amino acids, eight vary by 10–20%, and isoleucine, cystine and tryptophan have 22%, 45% and 75% differences respectively. These differences may, however, be more apparent than real. Examination of the data shows that Reithel & Robbins (1967) corrected aspartic acid, serine, threonine, glutamic acid, alanine, valine, methionine, isoleucine and tyrosine by extrapolation to zero time. Such corrections should only be necessary for serine, threonine, cystine and tyrosine (Moore & Stein, 1963). We suggest that these corrections are a source of error. For example, it is claimed that urease has a higher isoleucine than leucine content. However, as in our Table 1, the data show in all

Table 4. *Amino acid composition of jack-bean urease*

Nitrogen content determined by Dumas method on unhydrolysed urease was 16.65% (after ash and moisture corrections); nitrogen content calculated from amino acids recovered was 16.90%. Sulphur content determined by the Schöniger (1956) method was 1.15% (after ash and moisture corrections); sulphur content calculated from sulphur-containing amino acids was 1.2%.

	Composition	
	(g. of amino acid residue/100g. of dry protein)*	(nearest integral no. of residues/mol. of protein of mol. wt. 75000†)
Asp	11.08	72
Thr	6.09	45
Ser	4.53	39
Glu	10.72	62
Pro	5.31	41
Gly	4.85	64
Ala	5.78	61
Val	5.86	45
CyS	1.49	11
Met	3.23	19
Ile	7.64	51
Leu	8.57	57
Tyr	3.56	16
Phe	3.81	19
Lys	6.71	39
His	3.71	20
Arg	6.48	31
Trp	1.24	5
Amide	0.95‡§	45§
Total	100.67	697

* Best values from Tables 1, 2 and 3.

† Suggested molecular weight (see Bailey & Boulter, 1969).

‡ See the text.

§ Omitted from the total.

cases that more leucine than isoleucine was found in the hydrolysates. If the results recorded by Reithel & Robbins (1967) are corrected by the usual methods (see our Table 1), the agreement between the two analyses is much improved on a molar-ratio basis. Twelve of the amino acids are then in agreement within 10%, and two others are within 11%. The outstanding differences are in threonine (17%), proline (15%), cystine (45%) and tryptophan (75%). The destruction of 32% of the threonine within 22 hr. is likely to produce considerable error in estimation. Lack of the dialysis step may have made the half-cystine analysis of Reithel & Robbins (1967) artificially high; it has been reported (Sumner, 1951) that urease preparations contain a diffusible thiol impurity. The very high difference in tryptophan

values is an artifact caused by the low nitrogen recovery in the analysis of Reithel & Robbins (1967). As tryptophan is analysed independently of the other amino acids (Table 3), a fairer comparison is between the claimed content on a dry-weight basis. This comparison shows a difference of 30%, which, although high, is more reasonable.

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