XXIII. LABILE GLUTAMINE PEPTIDES, AND THEIR BEARING ON THE ORIGIN OF THE AMMONIA SET FREE DURING THE ENZYMIC DIGESTION OF PROTEINS.

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THAT the ammonia (amide-N) given by proteins on acid hydrolysis comes in part from acid amides present in the molecule has been recently proved by the isolation after enzymic digestion of *l*-asparagine from edestin [Damodaran, 1932] and of *d*-glutamine from gliadin [Damodaran *et al.*, 1932]. In the latter research the amount of *d*-glutamine present at various stages was estimated by the indirect method of Chibnall and Westall [1932]. At the end of the digestion of the gliadin the amount present was equivalent to 43 % of the total amide-N. More than half of this was lost during precipitation of bases with phosphotungstic acid and the final yield of crystalline *d*-glutamine was equivalent to only about 5 % of the total amide-N. Damodaran *et al.* [1932] concluded that the low yield was due in large part to the unstable nature of *d*-glutamine in aqueous solution. They pointed out however that the indirect determination of glutamine amide-N might include labile peptides of *d*-glutamine as well as this amide. Since Chibnall and Westall [1932] had shown that *l*-leucyl-*d*-glutamine was stable under the hydrolytic conditions employed it was suggested that these labile peptides might be those resembling *d*-glutamine itself in having a free amino-group in the γ -position with respect to the amide group.

That relatively large amounts of labile glutamine peptides might indeed be present under certain conditions in the enzymic digests of proteins followed from the unpublished work of Dr Jaaback carried out in this laboratory during 1932-33. Caseinogen was digested with trypsin, the products extracted with butyl alcohol and the distribution of glutamine amide-N determined by the indirect method of Chibnall and Westall. The monoamino-acid fraction, which would include both asparagine and glutamine, contained only one third of the glutamine amide-N present in the original digest. The other two thirds remained in the aqueous phase and if the validity of the amide hypothesis be assumed they must have represented labile peptides of glutamine of predominantly acidic or basic character.

Synthesis of glutamine peptides of the second type mentioned above and a comprehensive study of their properties were therefore clearly desirable, but on account of the probably labile nature of the γ -amide group the problem was not easy of solution by the older methods of peptide synthesis. By a fortunate coincidence however Bergmann and Zervas [1932] published about this time a new method which offered some likelihood of success. In practice this has been found excellent for the purpose and a neutral dipeptide *d*-glutaminylglycine, an acidic dipeptide *d*-glutaminyl-*d*-glutamic acid and a neutral tripeptide *d*-glutaminylglycylglycine have been prepared. In all three the amide group is as labile as in

d-glutamine itself and all exhibit to a lesser degree the abnormal reaction of this substance with nitrous acid during a determination of amino-N by the method of Van Slyke.

Since the method of Bergmann and Zervas [1932] permits the ready synthesis of *d*-glutamine from *d*-glutamic acid a sufficient amount of this amide has also been prepared to investigate for the first time a conclusion that can be drawn from the work of Damodaran *et al.* [1932] and of Jaaback (unpublished), that the ammonia which slowly accumulates during the enzymic digestion of proteins is due to the secondary decomposition of *d*-glutamine and *d*-glutaminyl peptides under the conditions employed and not to the presence of any specific amidase in the enzyme preparations. Opportunity has also been taken to investigate the hydrolysis of *d*-glutaminyl peptides by the yeast peptidases used in the digestion experiments, so that optimum conditions may be employed in future work.

Synthesis of glutamine peptides.

Treatment of *N*-benzylcarbonatoglutamic anhydride (I) with glycine ester on the lines of the synthesis of *d*-isoglutamine by Bergmann and Zervas [1932] gave analogously *N*-benzylcarbonato-*d*- α -glutamylglycine ethyl ester (II). Phosphorus pentachloride converted this into the γ -acid chloride (III) which reacted with ethereal ammonia to give *N*-benzylcarbonato-*d*-glutaminylglycine ethyl ester (IV). *N*-Benzylcarbonato-*d*-glutaminylglycine (V), obtained by hydrolysis of (IV), on hydrogenation in the presence of palladium yielded *d*-glutaminylglycine (VI). By substituting *d*-glutamic acid diethyl ester and glycylglycine ethyl ester for glycine ethyl ester in the above synthesis *d*-glutaminyl-*d*-glutamic acid¹ (VII) and *d*-glutaminylglycylglycine (VIII) were also prepared.

d-Glutamine was synthesised by a similar method. *N*-Benzylcarbonato-glutamic anhydride with sodium ethoxide gave *N*-benzylcarbonato-*d*-glutamic acid α -ethyl ester which was converted *via* the acid chloride to the γ -amide. Mild hydrolysis removed the ester group and the resulting *N*-benzylcarbonato-*d*-glutamine was catalytically reduced to *d*-glutamine. Experimental details of this synthesis are omitted, as one giving a better yield has since been described by Bergmann *et al.* [1933].

- I. $\text{CO} \cdot (\text{CH}_2)_2 \cdot \text{CH} \cdot \text{NH}(\text{Bz} \cdot \text{O} \cdot \text{CO}) \cdot \text{CO}$
 $\quad \quad \quad \underbrace{\hspace{1.5cm}}_{\text{O}}$
- II. $\text{CO}_2\text{H} \cdot (\text{CH}_2)_2 \cdot \text{CH} \cdot \text{NH}(\text{Bz} \cdot \text{O} \cdot \text{CO}) \cdot \text{CONH} \cdot \text{CH}_2 \cdot \text{CO}_2\text{Et}$.
- III. $\text{COCl} \cdot (\text{CH}_2)_2 \cdot \text{CH} \cdot \text{NH}(\text{Bz} \cdot \text{O} \cdot \text{CO}) \cdot \text{CO} \cdot \text{NH} \cdot \text{CH}_2 \cdot \text{CO}_2\text{Et}$.
- IV. $\text{CONH}_2 \cdot (\text{CH}_2)_2 \cdot \text{CH} \cdot \text{NH}(\text{Bz} \cdot \text{O} \cdot \text{CO}) \cdot \text{CO} \cdot \text{NH} \cdot \text{CH}_2 \cdot \text{CO}_2\text{Et}$.
- V. $\text{CONH}_2 \cdot (\text{CH}_2)_2 \cdot \text{CH} \cdot \text{NH}(\text{Bz} \cdot \text{O} \cdot \text{CO}) \cdot \text{CO} \cdot \text{NH} \cdot \text{CH}_2 \cdot \text{CO}_2\text{H}$.
- VI. $\text{CONH}_2 \cdot (\text{CH}_2)_2 \cdot \text{CH}(\text{NH}_2) \cdot \text{CO} \cdot \text{NH} \cdot \text{CH}_2 \cdot \text{CO}_2\text{H}$.
- VII. $\text{CONH}_2 \cdot (\text{CH}_2)_2 \cdot \text{CH}(\text{NH}_2) \cdot \text{CO} \cdot \text{NH} \cdot \text{CH}(\text{CO}_2\text{H}) \cdot (\text{CH}_2)_2 \cdot \text{CO}_2\text{H}$.
- VIII. $\text{CONH}_2 \cdot (\text{CH}_2)_2 \cdot \text{CH}(\text{NH}_2) \cdot \text{CO} \cdot \text{NH} \cdot \text{CH}_2 \cdot \text{CO} \cdot \text{NH} \cdot \text{CH}_2 \cdot \text{CO}_2\text{H}$.

N-Benzylcarbonato-*d*- α -glutamylglycine ethyl ester. 10 g. of *N*-benzylcarbonato-*d*-glutamic anhydride [Bergmann and Zervas, 1932] were dissolved in dry chloroform and 10 g. of glycine ethyl ester added. After 5 hours the solution was washed with dilute hydrochloric acid, dried with anhydrous sodium sulphate and the chloroform removed *in vacuo*. The residue was taken up in 10 ml. of absolute

¹ It is unfortunate that Bergmann and Zervas [1932] refer to glutamylglutamic acid as "Glutaminylglutaminsäure" instead of "Glutamylglutaminsäure" (*cf.* Chem. Zentr. 1923, III, 1084) as it will lead to confusion in German with the glutamine peptide glutaminylglutamic acid described in the present paper.

alcohol and left overnight in the ice-chest, when 6.5 g. of material crystallised out (47 % on the anhydride taken). Recrystallised from a small volume of alcohol it melted at 124°. (Found: N, 7.5 %. $C_{17}H_{22}O_7N_2$ requires N, 7.65 %. 0.174 g. required 4.75 ml. of 0.1 *N* NaOH; calculated, 4.75 ml.)

N-Benzylcarbonato-d-glutaminylglycine ethyl ester. 1.5 g. of the above ester in 30 ml. of dry chloroform were treated at -5° with 1 g. of pulverised phosphorus pentachloride. After 5 mins. of vigorous shaking the chloroform solution was decanted and evaporated at the pump for 10 mins. to remove free hydrochloric acid. It was then added to a cold saturated ethereal solution of ammonia. After allowing to stand for about an hour the precipitate was filtered off and after removal of the solvent was thoroughly triturated with water. The insoluble residue crystallised well from alcohol. Yield 0.9 g.; m.p. 167°. (Found: N, 11.5; amide-N (Sachsse) 3.8 %. $C_{17}H_{23}O_6N_3$ requires N, 11.5; amide-N, 3.8 %.)

N-Benzylcarbonato-d-glutaminylglycine. 2.9 g. of the above half amide half ester were dissolved in 100 ml. of alcohol, aqueous sodium hydroxide was added to the first blue colour of thymolphthalein, the solution cooled to 20° and 8 ml. of *N* NaOH added. After standing for 90 mins. a slight excess of acid was added, the solution filtered and the filtrate evaporated to 40 ml. at the pump. This was kept at 0° overnight when 2.1 g. of crystalline *N*-benzylcarbonatoglutaminylglycine were obtained, corresponding to a 78 % yield. The material was not further purified. (Found: N, 12.4; amide-N, 4.0 %. $C_{15}H_{19}O_6N_3$ requires N, 12.5; amide-N, 4.1 %.)

d-Glutaminylglycine. 2 g. of the above compound dissolved in 70 ml. of aqueous methyl alcohol were hydrogenated in the presence of palladium. The evolution of CO₂ stopped after 20 mins., when the solution was filtered from the catalyst, brought to neutrality with ammonia and evaporated at a temperature not exceeding 40° to 10 ml. The dipeptide crystallised out on addition of alcohol and was purified by recrystallisation from aqueous alcohol. Yield 0.7 g. (Found: C, 41.3; H, 6.4; N, 20.7; amide-N, 6.9 %. $C_7H_{13}O_4N_3$ requires C, 41.4; H, 6.4; N, 20.7; amide-N, 6.9 %.) For a 4.8 % solution in water the observed rotation was $\alpha_D^{18} + 3.65^\circ$, whence $[\alpha]_D^{18} = +76^\circ$.

d-Glutaminyl-d-glutamic acid. *N*-Benzylcarbonato-*d*- α -glutamyl-*d*-glutamic acid diethyl ester was prepared by the method of Bergmann and Zervas [1932], the yield being 65 % as against the quoted yield of 37 %. Following the procedure outlined above this was converted in 80 % yield into *N*-benzylcarbonato-*d*-glutaminyl-*d*-glutamic acid diethyl ester. Recrystallised from alcohol this melted at 181°. (Found: N, 9.1; amide-N, 3.0 %. $C_{22}H_{31}O_8N_3$ requires N, 9.0; amide-N, 3.0 %.) 2 g. of this amide ester were dissolved in 80 ml. of alcohol, a few drops of thymolphthalein solution added and then 4.6 ml. of *N* NaOH. The mixture was kept at 37° until an aliquot showed by back-titration with acid that the hydrolysis was complete (5–10 hours). 1.1 g. of *N*-benzylcarbonato-*d*-glutaminyl-*d*-glutamic acid were obtained. (Found: N, 10.1; amide-N, 3.3 %. $C_{18}H_{23}O_8N_3$ requires N, 10.3; amide-N, 3.4 %.) Reduction was carried out in the usual way. The dipeptide crystallised during the reduction and was separated from the catalyst by washing with a considerable volume of warm water. On evaporating the solution and washings *in vacuo* to a small volume, *d*-glutaminyl-*d*-glutamic acid crystallised out in 80 % yield. Purification was effected by dissolving in the smallest possible volume of water at 50°, evaporating *in vacuo* till the peptide began to crystallise and leaving overnight in the ice-chest. (Found: C, 43.6; H, 6.2; N, 15.5; amide-N, 5.1 %. $C_{10}H_{17}O_6N_3$ requires C, 43.7; H, 6.2; N, 15.5; amide-N, 5.2 %.) For a 4.8 % solution in *N* HCl the observed rotation was $\alpha_D^{18} + 0.63^\circ$, whence $[\alpha]_D^{18} = +15^\circ$.

d-Glutaminylglycylglycine. Glycylglycine ethyl ester, prepared from glycine ethyl ester by the method of Fischer and Fourneau [1901] reacted with *N*-benzylcarbonato-*d*-glutamic anhydride to give *N*-benzylcarbonato-*d*- α -glutaminylglycylglycine ethyl ester, m.p. 136°, in 54 % yield. (Found: N, 9.9 %. $C_{19}H_{25}O_8N_3$ requires N 9.9 %. 0.0198 g. required 3.15 ml. *N*/70 NaOH; calculated 3.30 ml.) The ester did not dissolve readily in chloroform but it was possible to prepare a supersaturated solution for the reaction with phosphorus pentachloride. Treatment with ammonia gave *N*-benzylcarbonato-*d*-glutaminylglycylglycine ethyl ester in 57 % yield. Recrystallised from alcohol this melted at 150–152°. (Found: N, 13.0; amide-N, 3.0 %. $C_{19}H_{26}O_7N_4$ requires N, 13.3; amide-N, 3.3 %). No crystalline product could be isolated after hydrolysis of the ester grouping, so methyl alcohol was added and the reduction carried out forthwith. The yield of the tripeptide was poor and there was insufficient material to effect complete purification. The best sample gave the following analysis. (Found: C, 40.8; H, 6.2; N, 19.3 %. $C_9H_{16}O_5N_4$ requires C, 41.5; H, 6.15; N, 21.5 %.)

d-isoglutamine. A sample of this prepared by the method of Bergmann and Zervas [1932] gave correct analytical figures for N and amide-N but did not give satisfactory results when titrated electrometrically [cf. Melville and Richardson, 1935], and it was necessary to purify the material by recrystallisation three times from aqueous acetone before sufficiently good constants could be obtained. Since the original sample gave an abnormally high amino-N (Van Slyke) value and yielded a considerable amount of ammonia on hydrolysis at p_H 4 it was suspected that the product was considerably contaminated with *d*-glutamine, *i.e.* the anhydride ring had opened to give both the α - and γ -amides, recalling the unexpected results obtained by Bergmann *et al.* [1934], who found that the condensation of benzylcarbonato-*L*-aspartic anhydride and *L*-tyrosine methyl ester gave the β - and not the expected α -dipeptide. Since the point is of importance in any synthesis utilising either of these anhydrides as starting material, further information as to the composition of Bergmann and Zervas' *isoglutamine* was desirable. The anhydride was treated with ammonia according to their directions and the product (5.8 g.) catalytically reduced. This material was crystallised from aqueous alcohol, two crops being obtained, weighing 2.1 g. and 0.5 g. respectively, a total yield of 89 %. The first crop on analysis gave a glutamine amide-N value of 10 % and the second of 35 % of the total amide-N. It was found that the *isoglutamine* could be purified either by heating its aqueous solution at 100° for an hour or by recrystallisation from aqueous acetone. Thus 1.4 g. of the first crop mentioned above were dissolved in water and heated in a boiling water-bath for one hour. On the addition of alcohol 1.1 g. of material which gave only 2 % of its total amide-N as glutamine amide-N were recovered. (Found: N, 19.2 %. $C_5H_{10}O_3N_2$ requires 19.2 %.) For a 5.4 % solution in water the observed rotation was $\alpha_D^{15} + 1.05^\circ$ whence $[\alpha]_D^{15} = +19.1^\circ$. Bergmann and Zervas give $[\alpha]_D^{25} = +21.1^\circ$, so that heating with water may have brought about slight racemisation.

One recrystallisation of another sample from aqueous acetone reduced the glutamine amide-N value from 12 % to 3 % of the total amide-N. From these results it is clear that a substance containing labile amide-N, undoubtedly *d*-glutamine, was present in the original *isoglutamine* preparations, and that when the anhydride ring of *N*-benzylcarbonato-*d*-glutamic anhydride was opened by ammonia both α - and γ -amides were formed in the proportion of about six to one.

It is reasonable to assume both from the figures given in the last column of Table I and from the data given in Table IV that in the case of the glutaminyll

peptides described if any of the γ -peptide derivatives were formed during the opening of the anhydride ring they were successfully removed during the crystallisations at subsequent stages. But the possibility that the condensation between anhydride and amino-acid ester does not always go completely in one direction should not be overlooked in future work.

The properties of d-glutamine and d-glutaminyl peptides.

As was expected the three peptides whose synthesis has just been described showed in lesser degree the abnormalities which distinguish *d*-glutamine from all other amino-acids. The results of an investigation are given in Table I. *d*-Glutaminylglycine and *d*-glutaminyl-*d*-glutamic acid both yield about 90 % of their amide-N as ammonia on hydrolysis at p_H 4, and although the abnormal reaction with nitrous acid is not so marked as with *d*-glutamine itself the values obtained are 35–50 % higher than calculated.

Table I. *Properties of d-glutamine and related compounds.*

	Amino-N (% total N)		Ammonia-N given on hydrolysis at p_H 4 (% total amide-N)
	Van Slyke	Calculated	
<i>d</i> -Glutamine	95	50.0	86.0
<i>d</i> -isoGlutamine	55	50.0	2.0
<i>l</i> -Asparagine*	50	50.0	1.0
<i>l</i> -Leucyl- <i>d</i> -glutamine*	33	33.3	2.8
<i>d</i> -Glutaminylglycine	49	33.3	91.0
<i>d</i> -Glutaminyl- <i>d</i> -glutamic acid	45	33.3	87.0
<i>d</i> -Glutaminylglycylglycine	32	25.0	65.0

* Values from Chibnall and Westall [1932].

These results emphasise the inaccuracy of the Van Slyke amino-N values obtained during the course of an enzymic digestion of proteins such as gliadin which yield relatively large amounts of ammonia and glutamic acid on acid hydrolysis. Further, the abnormalities due to glutamine and glutaminyl peptides cannot, as was assumed by Damodaran *et al.* [1932], be corrected by a determination of glutamine amide-N, since from the data available there appears to be no correlation between this value and the amino-N (Van Slyke) value. The abnormal Van Slyke values of these compounds may however explain a point raised by Calvery [1933]. When comparing amino-N (Van Slyke) with an alcohol (NaOH) titration he finds that in a digest of egg-albumin there is equivalence to within 3 % of amino- and carboxyl groups liberated and assumes that proline peptide bonds are not split to any marked degree. Egg-albumin contains, in terms of total N, 8.5 % glutamic acid-N and 7.3 % ammonia-N so that it is reasonable to assume that considerable amounts of glutamine and glutaminyl peptides are present in the later stages of digestion, whose effect on the Van Slyke values would compensate for the hydrolysis of proline peptides.

The stability of d-glutamine and glutamine peptides in aqueous solution at 37°.

The stability of *d*-glutamine at p_H values of 1.8, 7.0, 7.8 and 8.3, the optimum reactions for pepsin, aminopolypeptidase, dipeptidase and trypsin respectively, has been studied both in the presence and absence of these enzymes. (Tables II and III.) In aqueous solution at p_H 1.8 and 37° the breakdown was quite rapid, over 80 % of the total amide-N being liberated as ammonia in two days. At p_H 7.8 and p_H 8.3 approximately 40 % breakdown had occurred in two days, while at p_H 7.0 this degree of splitting was attained after about 3 days. Similar

experiments with *l*-asparagine and *l*-leucyl-*d*-glutamine showed that they were almost completely stable under these conditions. Insufficient material was available to determine the stability of the labile glutamine peptides at different p_H values, but an experiment at p_H 8.3 showed that *d*-glutaminyl-*d*-glutamic acid was split at approximately the same rate as *d*-glutamine itself. No appreciable glutaminase activity was present in commercial preparations of pepsin and trypsin, or in a week-old preparation of yeast peptidase. It will be seen that these results offer strong presumptive evidence for the theory that the ammonia formed during the digestion of proteins by the above-mentioned enzymes comes from the spontaneous breakdown of *d*-glutamine and labile glutamine peptides.

It is to be noted that Grassmann and Mayr [1932] found that a fresh yeast autolysate exhibited glutaminase activity. None was observed in the week-old preparation used in the present case, owing either to the fact that this enzyme is rapidly inactivated in ice-cold solution or to the different strains of yeast employed in English and German breweries.

Chibnall and Westall [1932] showed that when *d*-glutamine in aqueous solution was heated at 100° for 3 hours at p_H 4 not only was there a large liberation of ammonia from the amide group but also a fall in the amino-N (Van Slyke) from 190 to 10–20 % of the theoretical value, suggesting that the liberated glutamic acid had been rapidly transformed into pyrrolidonecarboxylic acid. Since breakdown of *d*-glutamine in aqueous solution also takes place at 37° it was necessary to find out what effect this might have on the titrimetric technique used to follow the enzymic hydrolysis of the glutaminyl peptides, for the production of glutamic acid would lead to an increase in the acetone (NaOH) titre whereas its transformation into pyrrolidonecarboxylic acid, with no free amino-group, would leave matters undisturbed.

Actually an experiment at p_H 7.0 in which aliquots were taken at the same time intervals for estimation of ammonia and for acetone titration showed that there is no appreciable increase in titre (Table III). This is confirmed by the results of Grassmann and Mayr [1932] who studied the action of asparaginase on *d*-glutamine at p_H 8.2 and found no significant difference in titre after 48 hours, during which time, as can be seen from Table II, nearly 40 % of the *d*-glutamine

Table II. *Stability of d-glutamine at p_H values of 1.85, 7.83 and 8.3.*

The *d*-glutamine used contained 18.7 % N. p_H values were checked at the hydrogen electrode. All solutions were made up to 25 ml. and 5 ml. aliquots taken for analysis. Temperature 37°. The ammonia liberated was estimated by means of the micro-apparatus of Parnas and Heller [1924] and is expressed in ml. of *N*/70 HCl.

At p_H 1.85 Solution A contained 0.0493 g. of *d*-glutamine and Solution B 0.0497 g. of *d*-glutamine + 0.05 g. of pepsin. Buffer solution *M*/10 glycine—HCl.

At p_H 7.83 Solution C contained 0.0468 g. of *d*-glutamine in *M*/20 phosphate buffer.

At p_H 8.3 Solution D contained 0.0506 g. of *d*-glutamine and Solution E 0.0505 g. of *d*-glutamine + 0.05 g. trypsin in *M*/20 barbitone (veronal) buffer.

At p_H 1.85				At p_H 7.83		At p_H 8.3		
Time in hours	NH ₃ liberated			Time in hours	NH ₃ liberated Solution C	Time in hours	NH ₃ liberated	
	Solution A	Solution B					Solution D	Solution E
5	0.98	0.95		20	0.99	18	0.80	0.79
25	3.27	3.23		46	1.93	42	1.74	1.71
49	4.14	4.14		68	2.27	66	2.41	2.42
100 % splitting	4.70	4.73		—	4.46	—	4.85	4.84

Table III. *Stability of d-glutamine at p_H 7.05.*

Solution A contained 0.0518 g. of *d*-glutamine made up to 15 ml. with *M*/40 phosphate buffer. 2 ml. aliquots were used for titration in acetone with 0.022 *N* alcoholic NaOH and for the estimation of free ammonia. Solution B contained 0.0396 g. of *d*-glutamine, Solution C 0.0402 g. of *d*-glutamine + 2 ml. of a week-old neutral autolysate of yeast in *M*/20 phosphate buffer. 5 ml. aliquots were used for analysis. Ammonia liberated is expressed in ml. of *N*/70 HCl.

Solution A			NH ₃ liberated		
Time in hours	Acetone titre	NH ₃ liberated	Time in hours	Solution B	Solution C
0	5.20	0.02	24	0.52	0.47
23	5.15	0.53	90	1.62	1.58
71	5.15	1.38	142	2.23	2.19
100 % splitting	—	3.3	—	3.8	3.87

must have been decomposed. It appears therefore that during the breakdown the glutamic acid must have been rapidly transformed into pyrrolidonecarboxylic acid.

Action of yeast peptidases on glutamine peptides.

Insufficient glutaminyl peptides were available to work out accurately a full *p_H*-activity curve with the mixture of peptidases obtained by plasmolysis of yeast with ethyl acetate and autolysis at *p_H* 6.5 [Grassmann, 1927]. Comparative studies have been made at *p_H* 6.3, 7.0 and 7.8 on the peptides *d*-glutaminylglycine, *d*-glutaminyl-*d*-glutamic acid and *l*-leucyl-*d*-glutamine with *dl*-leucylglycine as control. About 0.125 millimol. of the peptide was dissolved in 0.025 *M* phosphate buffer (at *p_H* 7.8, 0.05 *M* isoglutamine buffer solution was used [cf. Melville and Richardson, 1935]) and the calculated volume of 0.2 *N* NaOH added to bring the peptides to the same *p_H*. The requisite amount of enzyme solution, about 0.4 ml. which had previously been brought to the buffer *p_H*, was added and the solution made up to 10 ml. with buffer. All *p_H* values were checked at the hydrogen electrode. The hydrolyses were carried out at 37° and were followed by titration in 90 % acetone [cf. Willstätter and Waldschmidt-Leitz, 1921], using 0.022 *N* alcoholic NaOH as titrating agent and thymolphthalein as indicator. An experiment with the crude tripeptide *d*-glutaminylglycylglycine at *p_H* 7.0 showed that it was split at least as rapidly as *dl*-leucylglycylglycine.

Table IV. *Action of yeast peptidases on peptides of glutamine.*

Dipeptide	<i>p_H</i>	Wt. in g.-millimols	ml. of 0.2 <i>N</i> NaOH	Increase in titre				100 % splitting
				30 mins.	75 mins.	135 mins.	195 mins.	
<i>d</i> -Glutaminylglycine	6.3	0.126	—	0.60	0.90	1.00	—	1.14
	7.05	0.144	0.24	1.0	1.1	1.15	—	1.31
	7.8	0.119	0.40	0.20	0.70	1.0	—	1.08
<i>d</i> -Glutaminyl- <i>d</i> -glutamic acid	6.3*	0.131	0.65	0.75	0.90	1.10	—	1.19
	7.05	0.131	0.80	0.25	0.65	0.95	—	1.19
	7.8	0.124	1.0	0.10	0.35	0.50	—	1.08
<i>l</i> -Leucyl- <i>d</i> -glutamine	6.3	0.138	—	0.30	0.80	1.15	—	1.25
	7.05	0.133	—	0.20	0.55	0.95	—	1.21
	7.8	0.141	0.18	0.25	0.70	1.10	—	1.28
<i>dl</i> -Leucylglycine	6.3	0.259	—	0.10	—	—	0.95	1.18
	7.05	0.256	—	0.15	0.40	—	1.10	1.16
	7.8	0.253	0.30	0.20	0.70	1.05	—	1.10

* 1 ml. of enzyme was used instead of 0.4 as in the other cases.

The results given in Table IV show that all the peptides are rapidly split and suggest that p_H 7.0 or a little lower is the best reaction for the scission of peptides of *d*-glutamine with yeast peptidase, particularly as at this p_H the breakdown of *d*-glutamine itself is considerably retarded.

SUMMARY.

The three peptides *d*-glutaminylglycine, *d*-glutaminyll-*d*-glutamic acid and *d*-glutaminylglycylglycine have been synthesised by the method of Bergmann and Zervas [1932]. They are readily hydrolysed by yeast peptidases.

As was expected, they exhibit the labile characters of *d*-glutamine itself, *i.e.* they give abnormally high amino-N (Van Slyke) values and are unstable in aqueous solution at 100°. A determination of glutamine amide-N on an enzymic digest of a protein by the method of Chibnall and Westall [1932] therefore may include glutaminyl peptides as well as glutamine itself.

The stability of *d*-glutamine and glutaminyl peptides in aqueous solution at 37° and various p_H values has been determined. All the amide-N is hydrolysed to ammonia in 4–9 days, and the glutamic acid set free is transformed into pyrrolidonecarboxylic acid. The bearing that these observations have on the origin of the ammonia liberated during the enzymic digestion of proteins and also on the titrimetric technique generally used to follow the course of the digestion is briefly discussed.

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REFERENCES.

- Bergmann and Zervas (1932). *Ber. deutsch. chem. Ges.* **65**, 1192.
—— ——— and Salzmänn (1933). *Ber. deutsch. chem. Ges.* **66**, 1288.
—— ——— and Schleich (1934). *Z. physiol. Chem.* **224**, 17.
Calvery (1933). *J. Biol. Chem.* **102**, 73.
Chibnall and Westall (1932). *Biochem. J.* **26**, 122.
Damodaran (1932). *Biochem. J.* **26**, 235.
—— Jaaback and Chibnall (1932). *Biochem. J.* **26**, 1704.
Fischer and Fourneau (1901). *Ber. deutsch. chem. Ges.* **34**, 2868.
Grassmann (1927). *Z. physiol. Chem.* **167**, 202.
—— and Mayr (1932). *Z. physiol. Chem.* **214**, 185.
Melville and Richardson (1935). *Biochem. J.* **29**, 187.
Parnas and Heller (1924). *Biochem. Z.* **152**, 1.
Willstätter and Waldschmidt-Leitz (1921). *Ber. deutsch. chem. Ges.* **54**, 2988.