Botany. — *Amino acid Breakdown by Aspergillus niger.* By A. GORTER. (Communicated by Prof. J. C. SCHOUTE.)

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Introduction.

Little is known respecting the way in which amino acids are broken down by fungi. VAN WAESBERGHE (11) demonstrated that l(—)aspartic acid can be deaminated by Aspergillus niger, in which process oxygen is taken up, and MOOI (8) found that dl-alanine can also be deaminated by this fungus.

On the other hand, of recent years a number of enzymes have been found, which play a part in the amino acid breakdown by animal tissues and bacteria. The most important of these enzymes are given in Table I.

TABLE I.

Name	Reaction	Experimental conditions	Occurrence	Author
Aspartase I and II	l(—)aspartic acid $\rightleftarrows$ NH_3 + fumaric acid	Opt. pH: 6.0—7.5 Temp.: 37° anaerobic	Various bacteria	VIRTANEN and TARNANEN (10) GALE (4)
d-amino-oxidase	d-amino acid + $\frac{1}{2}$ O_2 $\rightarrow$ NH_3 + keto acid	Opt. pH: 8.5—9.0 Temp.: 37° aerobic	Kidney and liver extracts	KREBS (7) WARBURG and CHRISTIAN (12)
l-amino-oxidase	l-amino acid + $\frac{1}{2}$ O_2 $\rightarrow$ NH_3 + keto acid	Opt. pH: 7.4 Temp.: 37° aerobic	Slices of liver and kidney; bacteria	KREBS (7)
glutamic acid dehydrogenase	l(+)glutamic acid + H_2O—2 H $\rightleftarrows$ NH_3 + keto-glutaric acid	Opt. pH: 7.4 Temp.: 30° with as H_2 acceptor: methylene blue	Extr. of animal and plant tissues; bacteria; yeast	VON EULER et al. (3)
aminopherase	l-amino acid′ + keto acid′ $\rightleftarrows$ keto acid″ + l-amino acid″	Opt. pH: 7.4 Temp.: 37° aerobic and anaerobic	extr. of muscle tissue; bacteria; plant tissue	BRAUNSTEIN and KRITZMANN (1,2)

I have now investigated whether one or more of these enzymes might be of importance in the breakdown of amino acids by Aspergillus niger. A more detailed survey of the results obtained will be published shortly (5).

§ 1. *Methods.*

All the experiments were carried out with *starved fungus mats.* These

are mats which are reared on a culture-solution (RAULIN's solution was used in every case), and then placed on water for 10—20 hours. By this method not only the synthetic reactions were suppressed as far as possible and the breakdown reactions were promoted, but, moreover, starved mats can decompose amino acids more easily than when not starved, as VAN WAESBERGHE (l.c.) has demonstrated.

After the starvation, the water used for this was replaced by a phosphate buffer to which was added the amino acid to be examined, that had been brought to the same pH beforehand.

The culture of the mats, the starvation and the experiment itself was carried out in flasks in which the quantity of oxygen absorbed, and of carbon dioxide produced, could be determined at the same time, during the experiment. For this purpose a certain quantity of alkali was put into the tube A of the flask employed (Fig. 1), in which the carbon dioxide was absorbed which could be determined after the experiment. During the experiment the oxygen uptake caused a decrease in volume, which was measured in a gas-burette connected with the flask.

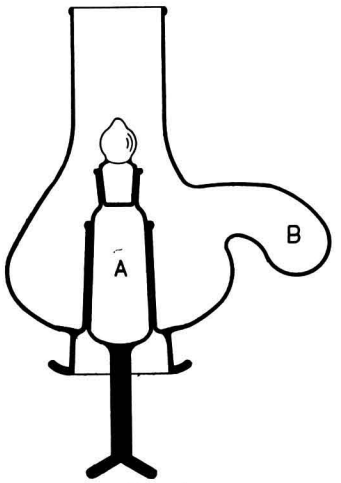

Fig. 1.

The substrate solution could be added when desired from the side-tube B, as is also usual with the Warburg method. The volume of the flasks was about 100 ml; during the experiment they were shaken in a thermostate at 25°, after being filled with O_2. A number of fungus mats was always investigated at the same time in order to eliminate the physiological variability as far as possible.

The starved mats show on a phosphate buffer also a respiration of their own; this must be subtracted from the respiration on phosphate buffer plus substrate in order to obtain the actual O_2 uptake and CO_2 production brought about by the substrate.

§ 2. *Deamination of aspartic acid.*

In the first place I investigated the influence of the pH on the rate of deamination. For this purpose the starved mats were brought on phosphate buffers of different pH, to which l(—)aspartic acid had been added to a final concentration of 0.025 mol. As a measure of the deamination rate, the quantity of NH_3 produced during the first 4 hours was determined. It appears now, from Table 2, that the results of the various experiments differ considerably, but that there is a distinct optimum at pH 2—4. For practical reasons the following experiments were always carried out at pH 4.5—5.0.

TABLE 2. Aspergillus mats reared for 2 days and thereafter starved for 12 hours. For pH 1.0 and 1.5: KCl/HCl buffer; pH 2.0 and 4.0: KH_2PO_4/H_3PO_4 buffer; pH 6.0 and 8.0: Na_2HPO_4/KH_2PO_4 buffer, always 0.1 mol. + 0.025 mol. l(—)aspartic acid. With experiment 63 and 65 10 ml buffer, and shaken in O_2 atmosphere; with experiment 66 20 ml buffer, not shaken, in air atmosphere. Temperature 25°. The figures show mg NH_3-N per mat, formed after 4 hours.

Exper. nr. \ pH	1.0	1.5	2.0	4.0	6.0	8.0
63	—	—	0.83	0.59	0.43	0.0
65	0.0	—	1.92	0.91	—	—
66	0.0	0.42	1.40	1.28	0.83	0.21

It is remarkable that the deamination proceeds so rapidly at so low a pH of the buffer solution, whereas the deamination enzymes described, all have a much higher pH optimum (Cf. Table 1). It is true that the pH *in* the cells is different, and probably higher, than in the surrounding buffersolution, while permeability and further transformations of the deamination product also play a part. Yet it is improbable that the pH, *in* the cell, will be near 7.5, for at this pH of the surrounding solution there is hardly any more deamination at all. This is an argument against the activity of the enzymes above-mentioned.

When the deamination and oxygen uptake were measured during the experiment it appeared that at first amino acid disappeared without much O_2 being taken up. Not until later is there a relative increase in the O_2 uptake (Table 3); and after complete deamination approx. 3—4 atoms of oxygen were taken up; VAN WAESBERGHE (l.c.) had already found a value of about 3 atoms. If the breakdown process be continued longer

TABLE 3. Aspergillus mats, 17 hours starved, thereafter on 10 ml phosphate buffer pH 5.0 with 0.024 mol. l(—)aspartic acid. After various lengths of time amino acid determined in solution. O_2 atmosphere. Temperature 25°. Figures mg per fungus mat.

Time in hours	Aspartic acid present mg	Aspartic acid disappeared mg	O_2 uptake ml 0°/76 cm	O_2 uptake in at. p. mol. asp. acid disappeared
0	31.7	—	—	—
2	23.7	8.0	0.53	0.8
4	15.9	15.8	2.24	1.7
6	12.9	18.8	4.37	2.8
10	5.8	25.9	6.67	3.0
24	2.3	29.4	9.82	4.0

the oxygen uptake proceeds but slowly, so that when 3—4 at. of oxygen per mol. aspartic acid has been taken up, the oxidation may be regarded as ended, the CO_2 formed being then approx. 2—2.6 mol. There is thus never complete oxidation. Moreover, the experiment shows that at first deamination proceeds more rapidly that the subsequent oxidation of the deamination product.

§ 3. *Deamination of other amino acids.*

With respect to a number of other amino acids I now investigated whether they were deaminated under the same experimental conditions as aspartic acid, i.e. at pH 4.5—5.0 and in O_2 atmosphere. This proved to be the case with glycocoll, d(—)- and l(+)alanine; dl-aspartic acid; l(+)glutamic acid, dl-leucine and dl-isoleucine; other amino acids were not investigated. None of the amino acids mentioned was oxidised completely, as is shown in Table 4. The deamination itself was complete, except in the case of dl-leucine and dl-isoleucine.

TABLE 4. Starved Aspergillus mats on phosphate buffer pH 4.5 — 5.0 + 0.025 mol. amino acid. O_2 uptake and CO_2 output calculated per mol. transformed amino acid. Averages of a number of experiments.

Substrate	O_2 uptake in at. p. mol. substrate	CO_2 production in mol. p. mol. substrate
l (—) aspartic acid	3.4	2.2
dl-aspartic acid	3.0	—
l (+) alanine	3.4	—
d (—) alanine	3.0	1.5
dl-alanine	3.1	—
glycocoll	1.5	0.8
l (+) glutamic acid	5.3	2.5
dl-leucine	5.1	—
dl-isoleucine	4.3	—

With the amino acids examined the deamination proceeded as a rule in the same way as with aspartic acid, i.e., at first the amino acid disappeared, while but little oxygen is taken up; later the oxygen uptake increased proportionately. The value found for the oxygen uptake and the carbon dioxide output are no indication respecting the way in which the amino acids are broken down or respecting the enzymes which are active thereby.

This last was now further studied by examining what was the first product of the deamination.

§ 4. *The enzymes which play a part in the deamination.*

When *aspartase* plays a part in the aspartic acid breakdown the deamination must also take place anaerobically, and fumaric acid must be demonstrable. This was not the case, however; in nitrogen atmosphere there is no deamination at all. It is possible that the permeability will be much reduced in nitrogen atmosphere and the aspartic acid assimilation therefore retarded, since Aspergillus is a highly aerobic organism. In an aerobic experiment any fumaric acid formed may disappear, however, by oxidation, and therefore Na-arsenite was added to inhibit the oxidation. Fumaric acid was determined according to the method of HAHN and HAARMANN (6) by precipitation as mercuric salt, but in not a single experiment could fumaric acid be demonstrated as a product of the aspartic acid breakdown.

From this it may be concluded that aspartase does not play any part in the breakdown of aspartic acid by Aspergillus.

In the same way I investigated whether the keto acids, which should be produced by the oxidative deamination by means of *amino-oxidases,* could be demonstrated. With aspartic acid the keto acid is oxalo-acetic acid, and with alanine pyruvic acid, for both substances there is a very sensitive colour reaction, as worked out by STRAUB (9). To inhibit a further oxidation of the keto acids Na-arsenite was again used; this substance, according to KREBS (l.c.) does not inhibit the deamination itself. It now appeared that, as a product of the deamination of l(—)aspartic acid, oxalo-acetic acid could never be demonstrated, but pyruvic acid was, although in very small quantities. This was also the case with the inhibited oxidation of dl-alanine; it was not investigated in the case of the other amino acids. Pyruvic acid was also produced, however, with the basal metabolism of the Aspergillus mats, when Na-arsenite was added.

According to these experiments it is thus improbable that an amino-oxidase plays any part, since the keto acids would then be produced in larger quantities. As further argument against the action of an oxidase is that the l- and the d-component of aspartic acid and alanine are deaminated exactly alike (Cf. Table 4), whereas for the oxidation of the d-component another enzyme must be active than for the l-component. The d-amino-oxidase is also active in tissue *extracts,* but it appeared that a ground Aspergillus mat was no more able to deaminate the d-component of aspartic acid and alanine than the l-component. Finally it appeared that glycocoll was easily deaminated, whereas this substance is not affected by the amino-oxidases.

The conclusion from the above experiments is, thus, that an amino-oxidase does not occur in Aspergillus niger, or in any case, does not play any part in the amino acid oxidation under the experimental circumstances.

This oxidation is closely connected with the cell-respiration, for with all the methods by which this latter is inhibited or suppressed (addition

of Na-arsenite, grinding, working in N_2 atmosphere) the amino acid oxidation is also inhibited, so that the amino acids are probably oxidised with the help of the ordinary cell-respiration enzymes.

That the *glutamic acid dehydrogenase* might play a part in the oxidation of l(+)glutamic acid is very improbable, for a ground fungus mat cannot oxidise this amino acid with methylene blue as H_2 acceptor. Now, it is very well possible that Aspergillus contains the apodehydrase which is activated by the addition of co-dehydrase I or II, as VON EULER et al. (3) found in a number of animal and plant tissues, but this implies nothing after all regarding the action of the enzyme under my experimental conditions, which are very favourable for the fungus used.

Lastly I investigated whether an aminopherase might be active, that is, whether a "transamination" between amino acid and keto acid might take place by the activity of the fungus. These experiments are not yet completed, but no such transamination has been obtained under various conditions indicated as being favourable. As an example, Table 5 gives the result of an experiment with keto-glutaric acid and alanine, from which it appears that neither glutamic acid nor pyruvic acid was formed at all.

TABLE 5. Starved Aspergillus mats on 10 ml phosphate buffer pH 7.4 in N_2 atmosphere. Addition 1 ml 0.2 % Br-acetate solution. Temp. 25°. Concentration in mol. per mat.

Substrate	l (+) alanine	ketoglutaric acid	l (+) alanine + keto-glutaric acid
Initial conc.	0.172	0.228	0.172 + 0.228
Alanine disappeared	0.024	—	0.009
Pyruvic acid formed	0.000	0.000	0.000
Glutamic acid formed	0.043	—	0.000

Summary.

The various enzymes hitherto known for the amino acid breakdown have no part in the deamination of amino acids by starved mats of Aspergillus niger. With this organism the oxidative deamination proceeds best at pH 2—4 of the surrounding solution. It is closely coupled with the ordinary cell-respiration, and is probably caused by "unspecific" oxidation enzymes.

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