A Study of Transamination System in *Fusarium*

A wide variety of transamination reactions have been studied in various organisms since BRAUNSTEIN and KRITZMAN[1] first discovered their importance. CAMMARATA and COHEN[2], HIRD and ROWSELL[3] and ROWSELL[4] have studied the scope and location of transamination systems in various animal tissues. FELDMAN and GUNSALUS[5] have shown that in some common bacteria transamination occurs between α-ketoglutarate and some 13 amino-acids resulting in the formation of glutamic acid. MILLBANK[6] demonstrated the occurrence of transaminase systems in the alga *Chlorella*, and WILSON, KING, and BURRIS[7] in some higher plants. In fungi, ROINE[8] working with *Torulopsis utilis* extracts showed that valine, leucine and isoleucine could serve as amino-group donors for α-ketoglutarate. Later FINCHAM[9] in *Neurospora crassa* and BIGGER-GEHRING[10] in *Saccharomyces fragilis* showed that some 10–11 amino-acids undergo transamination with α-ketoglutarate in cell-free extracts of the organisms.

In our laboratory, while some experiments on the nitrogen metabolism of *Fusaria* were in progress, it became necessary to know something about the scope of transamination reactions in the synthesis of glutamic acid by *Fusarium lycopersici*. The present communication embodies the results of this investigation.

Fusarium lycopersici, strains no. 5412 and 5414 were obtained from the Swiss Federal Institute of Technology (by courtesy of Dr. H. KERN). They were grown in 250 ml capacity Erlenmeyer flasks on 50 ml of a modified Richard's medium with an optimal micronutrient solution[11] without shaking. Inoculum was obtained in the form of conidia from peptone-yeast extract-agar slants. The flasks were incubated at 25°C for 72 h in dark. At the end of the incubation period, the mycelium was filtered on a Buchner funnel, washed thoroughly with glass-distilled water and sucked quite dry. After removing most of the water, the mycelium was weighed and immediately chilled to – 4°C in a glass container. After mixing alumina powder[12] (approximately twice the weight of mycelium), the whole mass was quickly ground to a fine paste in a chilled mortar after wetting with cooled *M*/15 phosphate buffer of pH 7·4. Grinding time did not exceed 5 min. The paste was extracted with 5 times its volume of phosphate buffer at about 2°C. The creamy mass was centrifuged at 2000 *g* at 2°C in a refrigerated centrifuge. The supernatant solution was dialyzed against 50 times *M*/15 phosphate buffer (pH 7·4) at 2°C for 8 h with stirring. The buffer was replaced by a new lot every 2 h. The extract so obtained contained about 0·5–0·7 mg N/ml.

For qualitative assay of transaminase activity in the extract, incubations were made in 15 ml centrifuge tubes at 37°C. The incubation mixture consisted of 0·4 ml dialyzed enzyme extract with 10 μM of amino-acid and 10 μM of α-ketoglutarate in a total volume of 1·0 ml at pH 7·4. In cases where L-isomer of the amino-acid was not available, 20 μM DL-isomer was used. 2 controls were kept with each series of experiment, one without α-ketoglutarate and one without enzyme. Incubation time was 6 h. At the end of this period, the tubes were placed in a boiling water bath for 3 min to end the reaction and later centrifuged to remove the precipitated proteins. The formation of glutamate in the incubation mixture was demonstrated with the help of paper chromatography. 1 μl of the supernatant was applied in duplicate to Whatman no. 1 filter paper and chromatographed using phenol-water (pH 12·0) and *n*-butanol, acetic acid, water (50:10:40) as solvent systems respectively. Amino-acid spots were revealed by spraying ninhydrin solution. Along with the incubation mixtures, chromatograms were also made simultaneously of different known quantities of glutamic acid and the amount of glutamate formed by transamination was roughly found by visual comparison with the spots of known glutamate concentrations. The Table gives the results of this investigation.

Tubes each containing in 1·0 ml: 0·4 ml dialyzed mycelial extract, 10 μM amino-acid and 10 μM α-ketoglutarate at pH 7·4 (*M*/15 phosphate buffer). Incubation at 37°C for 6 h. 0 = no transamination; + = about 1–2 μM glutamate; + + = 2–4 μM glutamate and + + + = 4–6 μM glutamate/ml.

Amino-acid	Concentration of glutamate
L-tyrosine	+
DL-threonine	0
L-arginine	0
L-hydroxyproline	0
L-ornithine	+
L-alanine	+ + +
DL-serine	0
L-methionine	+
L-*iso*leucine	+ +
DL-leucine	+ +
L-lysine	+
L-proline	+
L-cysteine	0
L-phenylalanine	+ +
L-tryptophane	+
L-citrulline	+ +
L-valine	+ +
DL-aspartic acid	+ + +
L-histidine	0
Glycine	0

Transamination occurred with tyrosine, ornithine, alanine, methionine, leucine, isoleucine, proline, phenylalanine, tryptophane, valine and aspartic acid out of a total of 20 different amino-acids tested. Maximum amount of transamination occurred with alanine at pH 7·4 and with aspartate at pH 8·5. The results obtained above are essentially similar to those obtained by FINCHAM[9] in *Neurospora crassa* and by BIGGER-GEHRING[10] in yeasts. Unlike these two organisms, however, ornithine in the case of *Fusarium* does not so readily undergo transamination with α-ketoglutarate.

In animals transaminases are localized in the particulate components of the cell[13]. According to the investigations of FINCHAM[9], however, transaminases seem to be present in the cytoplasm in *Neurospora*. To find out

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whether a similar state of affairs exists in *Fusarium*, the mycelial extract was made particle-free by either filtering it through kieselguhr[9] or spinning the extract at 19000 *g* in a refrigerated centrifuge for 30 min at 2°C. When such extracts were dialyzed and then tested for transaminase activity, essentially the same results were obtained as with ordinary extracts. This might mean that transaminases, at least in fungi, are not confined to the particulate fraction as in animal[5].

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Zusammenfassung

Aus *Fusarium lycopersici* wurde ein Enzympräparat gewonnen, welches Glutaminsäure durch Transaminierung aus α-Ketoglutarsäure bildete. Von den geprüften 20 Aminosäuren dienten die folgenden als Donatoren der Aminogruppe: Tyrosin, Ornithin, Alanin, Methionin, Leucin, Isoleucin, Prolin, Phenylalanin, Tryptophan, Valin und Asparaginsäure. Das Transaminasesystem scheint nicht an Strukturen des Cytoplasmas gebunden zu sein. Die Resultate stimmen im Prinzip mit jenen überein, welche bei *Neurospora* und bei Hefen gefunden wurden.

Specific Rotation of Phenylacetylglutamine Excreted by Cancer Patients and Normal Subjects

According to KÖGL[1], tumour tissue proteins contain substantial amounts of the 'unnatural' forms of some α-amino acids, particularly D-glutamic acid. These findings have been disputed[2], but recent work of HILLMANN *et al.*[3] lends further support to the possibility.

It was thought that the following procedure might throw some light on the question of the occurrence of D-glutamic acid in cancer patients.

When phenylacetic acid is ingested by humans, conjugation with glutamine occurs (other mammals utilise glycine[4]) and laevorotatory phenylacetylglutamine, $C_6H_5CONHCH(COOH)(CH_2)_2CONH_2$, is excreted in the urine[5]. If D- as well as L-glutamic acid is available for protein synthesis in cancer patients, it seemed possible that both 'natural' and 'unnatural' forms of glutamine might also occur in these patients. Thus the specific rotation of phenylacetylglutamine (PAG) excreted by cancer patients should be lower than that of the conjugate from normal subjects by an amount determined by the quantity of D-glutamine available for conjugation. Since phenylacetic acid in small doses is well-tolerated by humans[6] (PAG has been observed as a normal metabolite in humans[7]) it was decided to compare polarimetrically conjugates from normal persons and from cancer cases who ingested small amounts of phenylacetic acid.

In the experiment now reported, 2 male cancer patients and 1 male normal subject each received by mouth 1 g of phenylacetic acid (as sodium salt; 100 ml of bicarbonate solution) and the dose was repeated 3 h later. Urine was collected under toluene for 24 h from the start of the experiment. The urines were concentrated at 35–40°C *in vacuo*, acidified with sulphuric acid and extracted continuously with ethyl acetate following, with a few modifications, the method of THIERFELDER and SHERWIN[5]. The products, which were obtained as silky white flakes after one crystallisation from ethyl acetate, melted indefinitely at about 110°C and decomposed vigorously at ~ 120°C. Despite the similarity in melting point behaviour, nitrogen analyses (see Table, col. 3) showed that the substances were not pure PAG (for which calculated N% = 10·6), and manometric studies[8] (Table, col. 5) indicated that they contained appreciable amounts of urea. THIERFELDER and SHERWIN[5] obtained, in addition to pure PAG, a complex consisting of 1 mole PAG + 1 mole urea for which they recorded a specific rotation of − 14°. In our experience, it was found that without taking steps to remove urea before extraction of the urine with ethyl acetate, PAG products containing up to several moles of urea could be isolated.

For the present experiment, no attempt was made to eliminate urea from the once-crystallised products, the rotations of which were measured in distilled water at ~ 20°C. For calculation of the specific rotation [α], the weight of PAG in each product was estimated by subtracting the weight of urea as determined (a) by manometry and (b) by calculation from N% (assuming that the product contained PAG and urea only; no glucuronide

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Specific rotations of phenylacetylglutamine excreted by cancer patients and a normal subject

Subject	Condition	PAG complex from urine						
		Found N%	Calculated urea %	Manometric urea %	g complex/100 g water	α	$[\alpha]_D^{20}$ corrected for urea % as	
							calculated	determined manometr.
L	Myeloid leukaemia	16·5	16·4	—	2·0087	− 0·302	− 17·97	—
M	Teratoma of testis (operated). Extensive secondaries in para-aortic region and widespread deposits in lungs	21·1	29·2	29·4	1·9785	− 0·241	− 17·45	− 17·45
N	Normal	17·1	18·0	20·3	1·9646	− 0·285	− 18·34	− 18·17