Effect of L-Histidine on the Catabolism of Nitrogenous Compounds in *Aspergillus nidulans*

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It has been found that L-histidine is an extremely poor sole source of nitrogen for *Aspergillus nidulans* and is not a significant carbon source for this organism (Hynes, 1973; Polkinghorne and Hynes, unpublished). This paper reports the surprising finding that the presence of L-histidine in the medium prevents the utilization of many other nitrogen sources by this organism.

Growth tests on solid medium at 37 °C were carried out according to methods described previously (Hynes, 1973). It should be noted that there is always residual growth on medium lacking a nitrogen source ('nitrogen-free growth') presumably due to the presence of small amounts of nitrogen sources in the agar and solutions used. This permits a distinction to be made between inhibition of growth and poor utilization of nitrogen sources, and it should be emphasized that in none of the growth tests described here was there inhibition of growth by histidine.

Since histidine is a very poor source of nitrogen for wild-type *A. nidulans* it was possible to test for a decrease in the utilization of better nitrogen sources by the presence of histidine. It was initially found that growth of the wild-type strain (*biA1*, a biotin auxotroph) on medium containing 10^{-2} M-L-histidine together with 10^{-2} M-L-glutamate as the sole nitrogen sources was equivalent to the poor growth observed on medium containing 10^{-2} M-L-histidine as the sole nitrogen source. Subsequently it was found in similar tests that the utilization of the L-amino acids, arginine, valine, aspartate, phenylalanine, tyrosine, threonine, asparagine, tryptophan, glycine and ornithine, was also inhibited by histidine. Urea, hypoxanthine, uric acid and allantoin also were not utilized as nitrogen sources in the presence of histidine, while utilization of ammonium, nitrate, acetamide, formamide, proline and powdered-milk were not affected. Concentrations of histidine as low as 10^{-3} M gave similar effects to 10^{-2} M. D-Histidine reduced urea and L-glutamate utilization, but not to the same extent as L-histidine. The utilization of L-glutamate as a sole carbon source (with 10^{-2} M-ammonium as the nitrogen source) and the utilization of L-tyrosine and L-glutamate as sole carbon and nitrogen sources was greatly reduced by the presence of L-histidine in the medium. Histidine, however, had no effect on the utilization of the carbon sources glucose, sucrose, glycerol, galactose or acetate.

L-Leucine, like L-histidine, is a very poor sole nitrogen source for *A. nidulans* (Hynes, 1973). Therefore equivalent tests for the effects of L-leucine on nitrogen-source utilization could be made. L-Leucine did not affect growth on any other nitrogen sources.

Growth of wild type mycelium in L-histidine (10^{-2} M)-containing medium has been found to result in a very marked reduction (approximately 98 %) in the ability of mycelium to incorporate L-[^{14} C]glutamate (results not shown). This assay almost certainly largely reflects the activity of the acidic amino acid uptake system (Robinson, Anthony & Drabble, 1973). The presence of 10^{-3} M-L-histidine in the uptake assay was found to inhibit the incorporation

Table 1. *Effect of growth in the presence of L-histidine on urease and urate oxidase specific activities*

Growth conditions*		Specific activities†	
Initial incubation	Final incubation	Urease	Urate oxidase
NH ₄ ⁺	None	187	20
NH ₄ ⁺	Uric acid	715	84
NH ₄ ⁺	Uric acid + 10 ⁻³ M-L-histidine	182	77
NH ₄ ⁺	Uric acid + 10 ⁻² M-L-histidine	173	80
NH ₄ ⁺ + 10 ⁻² M-L-histidine	None	13	NT
NH ₄ ⁺ + 10 ⁻² M-L-histidine	10 ⁻² M-L-histidine	18	NT
NH ₄ ⁺ + 10 ⁻² M-L-histidine	No nitrogen source	431	NT
NH ₄ ⁺ + 10 ⁻² M-L-histidine	No nitrogen source + cycloheximide	37	NT

NT, Not tested.

* Mycelium of the wild-type strain (*biA1*) was grown in nitrogen-free glucose-minimal medium for 16 h (initial incubation) with the indicated additions. Then mycelium was either transferred to fresh glucose-minimal medium for a further 4 h (final incubation) or used directly (none). NH₄⁺ was added as 10⁻² M-ammonium tartrate. The concentrations of uric acid and cycloheximide were 0.25 mg/ml and 15 µg/ml, respectively; L-histidine concentrations were as indicated. Methods for growing mycelium have been described (Hynes, 1972).

† Crude extracts of mycelium were made in 10⁻¹ M-orthophosphate buffer pH 7.2, as described by Hynes (1972). Urate oxidase (EC. 1.7.3.3) was assayed by the method described by Hynes (1974). Urease was assayed by the phenol-hypochlorite method used for amidase assays (Hynes, 1970) with 10⁻² M-urea in 10⁻³ M-orthophosphate buffer pH 7.2 as substrate. Specific activities are expressed as nmol substrate converted (urate oxidase) or product formed (urease)/min/mg soluble protein.

of L-[¹⁴C]glutamate, as has been found by Robinson *et al.* (1973). Therefore the effects of histidine on glutamate utilization may be via inhibition of uptake and perhaps repression of the uptake system. The effects of histidine on other amino acid uptake systems has not yet been investigated.

Investigations have been carried out on a number of systems of nitrogen-source catabolism to determine the effects of histidine. L-Histidine was not found to relieve the toxic effects of thiourea, indicating that urea uptake is not affected (Dunn & Pateman, 1972). Growth in medium containing L-histidine led to very low levels of urease activity (Table 1). These levels were much lower than those present in mycelium grown in the presence of ammonium alone. Cycloheximide inhibited the rise in urease activity following transfer from L-histidine-containing medium to medium lacking L-histidine. This suggests that enzyme synthesis may be repressed by L-histidine. Experiments involving mixing of crude extracts failed to reveal the presence of an inhibitor of urease in extracts of L-histidine-grown mycelium, and histidine was not found to inhibit urease activity in crude extracts.

Histidine repression of urease synthesis probably also accounts for the effects of L-histidine on hypoxanthine, uric acid and allantoin utilization, since urease activity is required for their full catabolism (Scazzocchio & Darlington, 1968). In addition, however, 10⁻² M-L-histidine was found to relieve the inhibition of formation of green conidial pigment by 2-thioxanthine, indicating that either 2-thioxanthine uptake or xanthine dehydrogenase (EC. 1.2.3.2) activity or both are affected by histidine (for the basis of this test see Darlington & Scazzocchio, 1967). It is shown in Table 1 that L-histidine did not prevent the normal induction of urate oxidase, the second enzyme of purine degradation (Scazzocchio & Darlington, 1968). However, urate oxidase activity in crude extracts was inhibited by L-histidine (but only at relatively high concentrations: 35 % inhibition at 2.5 × 10⁻³ M and 55 % at 10⁻² M).

Is histidine or a catabolite of histidine responsible for the effects on the utilization of other nitrogen sources? The utilization of histidine as a nitrogen source by *A. nidulans* occurs chiefly via the enzyme histidase (histidine-ammonia lyase, EC. 4.3.1.3). A number of strains, altered in their abilities to produce histidase, have been isolated (Polkinghorne and Hynes, unpublished). All of these show similar effects of histidine on nitrogen-source utilization, indicating that it is unlikely that the degree to which L-histidine is catabolized via histidase affects the use of other nitrogen sources. Furthermore, urocanate, one of the products of histidase activity, is not a sole nitrogen source and does not affect the utilization of any sources of nitrogen. Ammonium, the other product of histidase activity, is known to repress many nitrogen catabolic systems (see Pateman, Kinghorn, Dunn & Forbes, 1973), but clearly ammonium production from L-histidine cannot account for the results since only some ammonium-repressible systems are affected and the sensitivity of urease to histidine repression is much greater than its sensitivity to ammonium repression (Table 1). In addition, L-histidine was found to inhibit the utilization of L-glutamate by strains containing the *gdhA10* lesion, which results in low NADP-dependent glutamate dehydrogenase activity and loss of sensitivity to ammonium repression (Arst & MacDonald, 1973; Kinghorn & Pateman, 1973). Therefore, it can be concluded that either L-histidine itself, or a L-histidine metabolite other than urocanate or ammonium, is responsible for these effects. Where inhibition of enzyme or uptake-system activity is involved it is most likely, of course, that L-histidine itself is responsible.

These results force us to the conclusion that *A. nidulans* possesses mechanisms for the preferential catabolism of L-histidine from mixtures of L-histidine and many other potential nitrogen sources. We can suggest no reasonable physiological advantage to the organism in possessing these mechanisms.

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