Such a close association between IAA oxidase and peroxidase can be visualized from the evidence presented by a number of workers[12–15]. This is also supported by the fact that a unique IAA oxidase enzyme has not been found, except in the tobacco root extracts[16].

[12] B. Z. Siegel and A. W. Galston, Science *157*, 1557 (1967).
[13] R. E. Stutz, Pl. Physiol. *32*, 31 (1957).
[14] P. M. Ray, Arch. Biochem. Biophys. *87*, 19 (1960).
[15] B. Darbyshire, Physiologia plant. *29*, 293 (1973).
[16] L. Sequeira and L. Mineo, Pl. Physiol. *41*, 1200 (1966).
[17] The research has been partly financed by a grant from the U.S. Department of Agriculture. One of us (RNC) is thankful to the CSIR, India, for financial assistance.

Summary. The IAA oxidase enzyme extracted from *Phaseolus mungo* hypocotyls appears to show allosteric behaviour with 2 sites, one representing the oxidase (site I) and the other peroxidase (site II). It is considered that the site I is concerned in the production of active IAA oxidation products, while site II merely acts to detoxify the excessive IAA.

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Inhibition of Cathepsin A Activity by the Potato Protease Inhibitor

Only a few of the natural inhibitors of intracellular proteolytic enzymes have so far been discovered. Nothing is known about the natural inhibitors of cathepsin A. Inhibitors of cathepsin B and C occur in the cytoplasm of liver cells[1] and in the hen's egg-white[2]. Cathepsin B_1 activity is inhibited by α_2-macroglobulin[3], the carbohydrate inhibitor associated with haptoglobin[4], and leupeptin[5]. Cathepsins D and E are inhibited by pepstatin[5,6]. A specific serum antibody inactivates cathepsin D[7] and cathepsin E is inactivated by an inhibitor isolated from *Ascaris lumbricoides*[8].

Our preliminary experiments showed that acid cellular proteases of a spleen homogenate were partly inhibited by the potato protease inhibitor (PPI) when tests of their activity on haemoglobin at pH 3.5 were made[9,10]. In these investigations, an attempt was made to determine which of these enzymes was inhibited by the PPI. Purified preparations of intracellular proteases and specific substrates were used for these experiments. Since, of all the intracellular proteases, only cathepsin A and particularly cathepsin D bring about the degradation of haemoglobin[11], particular attention was paid to those enzymes.

The PPI was obtained by the method previously described[10]. Cathepsin A and D were obtained from hen muscles according to the method given by Jodice et al.[11]. The source of cathepsin B_1 and C was 10% homogenate of ox spleen.

The effect of PPI in various concentration (0.0062 to 0.1 mg/ml) on cathepsin A (0.6 mg/ml) activity was tested according to the method of Jodice et al.[11]. The substrate was N-α-carbobenzoxy-L-glutamyl-L-tyrosine (6.2 m*M*) in a 0.1 *M* acetate buffer (pH 6.0). Incubation was carried out for 1 h at 37 °C. The reaction was stopped by adding TCA and the α-amine-nitrogen released was determined by the ninhidrin colorimetric method[12].

Inhibition of cathepsin A activity by various concentrations of potato protease inhibitor (PPI) measured on N-α-carbobenzoxy-L-glutamyl-L-tyrosine[a]

PPI, final concentration (mg/ml)	α-amino-nitrogen (m*M*/ml)	Activity (%)
0.100	2.3	1.0
0.050	5.8	2.5
0.025	61.0	27.1
0.012	120.0	53.3
0.00625	150.0	66.4
Control	225.0	100.0

[a] Final concentration: of enzyme, 0.6 mg/ml; of substrate, 6.2 m*M*.

The effect of PPI (0.1 mg/ml) on cathepsin B_1 activity was investigated according to the method described by Keilova and Tomašek[2]. The substrate was N-α-benzoyl-D,L-arginine-*p*-nitroanilide (1 mg/ml) in a 0.1 *M* phosphate buffer (pH 6.0) containing 1 m*M* EDTA and 25 m*M* cysteine-HCl for activation. Incubation was carried out for 1 h at 40 °C. The reaction was stopped by adding glacial acetic acid. The *p*-nitroaniline released was determined spectrophotometrically at 405 nm.

The effect of PPI (0.1 mg/ml) on cathepsin C activity was investigated by the same procedure as that used for cathepsin B_1 activity[2] except that glycyl-L-phenylalanine-*p*-nitroanilide (0.1 mg/ml) was used as substrate.

The effect of PPI (0.1 mg/ml) on cathepsin D (0.4 mg/ml) activity was determined by using as substrate urea-denaturated haemoglobin (10 mg/ml) in 0.04 *M* Britton and Robinson universal buffer (pH 3.5)[13]. Incubation was carried out for 1 h at 37 °C. The reaction was stopped by adding TCA, and the tyrosine released was determined by means of the Folin-Ciocalteu reagent[14].

The PPI inhibits cathepsin A activity (Table). The degree of cathepsin A activity inhibition depends on the PPI concentration. In the presence of the PPI in a final concentration of 0.1 mg/ml, approximately 1% of the activity of the enzyme remained. This inhibitor does not inhibit the activity of cathepsin B_1, C and D. Cathepsin A is inhibited by diisopropylofluorphosphate serine carboxy-

[1] J. Finkenstaedt, Proc. Soc. exp. Biol. Med. *95*, 302 (1957).
[2] H. Keilova and V. Tomašek, Biochim. biophys. Acta *334*, 179 (1974).
[3] P. M. Starkey and A. J. Barrett, Biochem. J. *131*, 823 (1973).
[4] O. Snellman and B. Sylvén, Experientia *30*, 1115 (1974).
[5] H. Ikezawa, T. Aoyagi, T. Takeuchi and H. Umezawa, J. Antibiot. *24*, 488 (1971).
[6] A. J. Barrett and J. T. Dingle, Biochem. J. *127*, 439 (1972).
[7] J. T. Dingle, A. J. Barrett and P. D. Weston, Biochem. J. *123*, 1 (1971).
[8] H. Keilova and V. Tomašek, Biochim. biophys. Acta *284*, 461 (1972).
[9] K. Worowski and T. Mariak, Acta Pol. pharm. *30*, 453 (1973).
[10] K. Worowski, Thromb. Diath. haemorr. *32*, 617 (1974).
[11] A. Jodice, V. Leong and J. Weinstock, Arch. Biochem. Biophys. *117*, 477 (1966).
[12] T. Devenyi and T. Gergely, *Analytische Methoden zur Untersuchung von Aminosäuren, Peptiden und Proteinen* (Akademiai Kiadó, Budapest 1968), p. 249.
[13] M. L. Anson, J. gen. Physiol. *20*, 265 (1937).
[14] O. Folin and V. Ciocalteu, J. biol. Chem. *73*, 627 (1927).

peptidase[15]. Thus it is probably sensitive to the action of the PPI as are many other proteolytic enzymes with serine active centres[10]. Cathepsin A as serine carboxypeptidase is not sensitive to the potato inhibitor of the pancreatic carboxypeptidases A and B which are metaloproteinases[16].

Cathepsin A acts chiefly on the products of partial haemoglobin degradation formed as a result of the action of cathepsin D[11]. Haemoglobin degradation by cathepsin A occurs most rapidly at pH 3.5, whereas when synthetic substrates are split by this enzyme, the optimum pH is 5.4[17].

Since the PPI does not inhibit cathepsin D activity, it would seem that the partial inhibition of haemoglobin degradation by the acid cellular proteases at pH 3.5 in the presence of the potato inhibitor, observed previously[9,10], depends on the inhibition of this cathepsin A inhibitor.

Summary. It was found that the protease inhibitor from the potato inhibits cathepsin A activity. This inhibitor does not inhibit the activity of cathepsin B_1, C and D.

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[15] A. J. LOGUNOV and V. N. OREKHOWICH, Biochim. biophys. Res. Commun. *46*, 1161 (1972).
[16] C. A. RYAN, J. biol. Chem. *249*, 2495 (1974).
[17] J. S. FRUTON, in *The Enzymes* (Eds. P. D. BOYER, H. LARDY and K. MYRBACK; Academic Press, New York 1960), vol. 4, p. 233.

Differences in RNA Content Related to Mating Type in *Ascobolus immersus*

Several mechanisms which reduce self-fertilizing and encourage reassortment of genetic differences through outbreeding have been described in fungi[1]; but, until now, very little is known of the physiological basis of mating type determination in these organisms.

In *Ascobolus immersus*, 2 alleles (A and a), at a single locus, determine mating type. This 2 allele system, which represents the simplest form of heteroallelism, is very widespread being found in all groups of fungi.

Previous reports from this laboratory[2–4] indicated that certain variations in mobility and RNA content are related to the differential residual genotype or genetic background of individuals; which suggested that *Ascobolus* might be a most interesting system to investigate this problem of sexual differentiation for which a dearth of information exists. The present paper reports data showing real differences in the relative proportions of stable RNA components in wild type strains of opposite mating types which are probably related to the genetic control of sexual development.

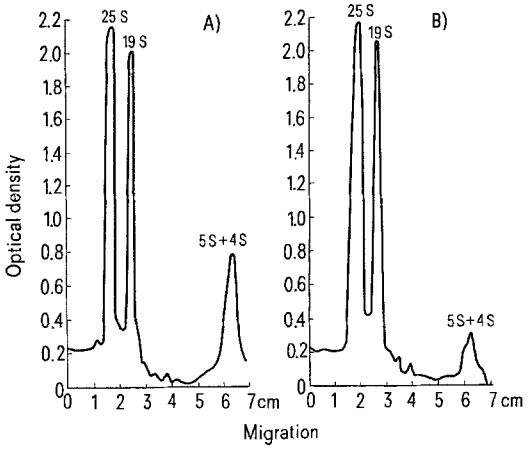

Fig. 1. 2.4% polyacrylamide gel electrophoretic pattern of total RNA isolated from *Ascobolus immersus* wild-type strains S2A (A) and S2a (B). Abscissa: distance migrated in cm; ordinate: optical density at 260 nm.

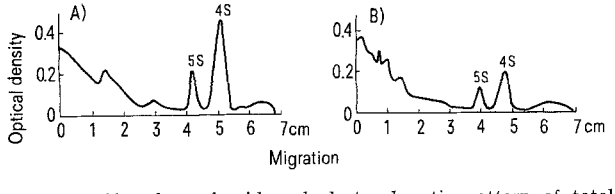

Fig. 2. 7.5% polyacrylamide gel electrophoretic pattern of total RNA isolated from *Ascobolus immersus* wild-type strains S2A (A) and S2a (B). Abscissa: distance migrated in cm; ordinate: optical density at 260 nm.

Materials and methods. The locally collected wild-type strain S2 of *A. immersus* was kindly supplied by Dr. J. R. BEAUDRY from this institution. RNA was isolated from the mycelium of the different strains by cold phenolic deproteinization; and the RNA profiles were obtained after polyacrylamide gel electrophoresis, as previously described[3].

Results. Figures 1 and 2 show the electrophoretic patterns of global RNA obtained from S2A (A) and S2a (B) in 2.4% and 7.5% polyacrylamide gels, respectively. One should expect that the relative proportions of stable RNA components to remain constant in both wild-type strains of opposite mating types, but it does not (Table). The 4S and 5S peaks are significantly higher for S2A than S2a, while all the other RNA populations are present in equivalent proportions. 5 different extracts from the same strains confirmed this apparent relationship between the mating type allele and the relative RNA distribution.

So as to get more information about such a relationship, one ascus presenting 8 wild-type ascospores was isolated in the progeny of the wild-type culture S2A X S2a. Each spore of the selected ascus yielded a strain which was backcrossed to the 2 parental strains so as to determine the exact mating type; and the RNA populations found in the mycelium produced by each of the 8 segregants were studied. Surprizingly, electrophoretic RNA profiles of wild-type strains which genotype is A are practically identical to those of the parental strain S2A (Figures 1A and 2A) and vice versa. Statistical

[1] J. R. S. FINCHAM and P. R. DAY, *Fungal Genetics* (Blackwell Scientific Publications, Oxford 1971).
[2] C. HAMELIN and G. H. COUSINEAU, Revue Can. Biol. *33*, 243 (1974).
[3] C. HAMELIN and G. H. COUSINEAU, Can. J. Genet. Cytol. *16*, 39 (1974).
[4] C. HAMELIN and G. H. COUSINEAU, Revue Can. Biol. *33*, 249 (1974).