# IMMUNOLOGICAL DIFFERENCES BETWEEN INDUCIBLE AND CONSTITUTIVE XANTHINE DEHYDROGENASES IN *ASPERGILLUS NIDULANS*

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## 1. Introduction

Mutants constitutive for xanthine dehydrogenase in *Aspergillus nidulans* belong to two classes. The first group, $oxp^r$, selected by resistance to the purine analogue oxoallopurinol (alloxanthine, 4,6-dihydroxypyrazolo-(3,4-d)pyrimidine) are also constitutive for urate oxidase. Mutants in the second group $apl^r$, (previously called *allp* [1], selected by resistance to the analogue allopurinol (4-hydroxypyrazolo-(3,4-d) pyrimidine) on minimal medium plus hypoxanthine as sole nitrogen source are not constitutive for urate oxidase [1, 2].

The xanthine-dehydrogenase-constitutive $apl^r$ mutants all map at one locus in linkage group VI [2]. In the present paper investigations on the identity of the enzyme(s) produced by the induced wild type and the constitutive $apl^r$ mutants are reported. It is shown that the $apl^r$ mutants when grown under non-inducing conditions produce a different enzyme from the one made by the induced wild type chain. When $apl^r$ mutants are induced with uric acid they produce both enzyme forms.

## 2. Materials and methods

### 2.1. *Strains*

Two strains were used, *bi*A-1 a translocation free biotin auxotroph and *bi*A-1 $apl^r$-7 (previously called *allp*-7) a spontaneous mutant, constitutive for xanthine dehydrogenase activity, derived from *bi*A-1. The properties of this mutant have been described [1]. Growth of the strains and preparation of mycelial extracts have been described in detail previously [3]. Xanthine dehydrogenase activity was assayed by following the reduction of cytochrome *c* at 550 nm in the presence of benzyl viologen on a Pye Unicam SP8000 spectrophotometer [4].

### 2.2. *Preparation of antisera*

Antisera were prepared in New Zealand White rabbits. Three intramuscular injections of mycelial extracts homogenized with Freund's adjuvant (Difco Laboratories) were given at weekly intervals following a control bleeding. The animals were bled one week after the final injection. Antisera were prepared against extracts of *bi*A-1 fully induced for xanthine dehydrogenase activity by growth for 20 hr on minimal medium plus uric acid (100 μg/ml) as sole nitrogen source and against extracts of *bi*A-1 $apl^r$-7 grown on minimal medium plus urea (5 mM) as sole nitrogen source for 20 hr. The antisera will be referred to as anti-wild type and anti-$apl^r$-7 respectively.

### 2.3. *Double diffusion plates*

1% Agar Noble in 0.1 M orthophosphate buffer pH 7.0 was used. The wells were punched with a number 1 cork borer. The capacity of each well was approximately 15 μl. Time of incubation at 25° varied from 16–48 hr.

### 2.4. *Staining for xanthine dehydrogenase activity*

Darlington [5] has shown that the precipitin lines of the xanthine dehydrogenase–antiserum complex can be stained for xanthine dehydrogenase activity in wild type *Aspergillus*. The following modified staining procedure was used [4]: hypoxanthine 0.5 mg/ml in 0.1 M pH 9.4 sodium pyrophosphate buffer, 2 ml; colchicine, 50 mg/ml in distilled water, 200 μl; 2-(*p*-

iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride 1.27 mg/ml in 0.1 M pH 9.4 sodium pyrophosphate buffer, warmed for 30 min and filtered before use, 5 ml. The plates were incubated at 25° with the assay mixture for 24 hr. They were then washed in distilled water for several hour and dried at 37° before photographing.

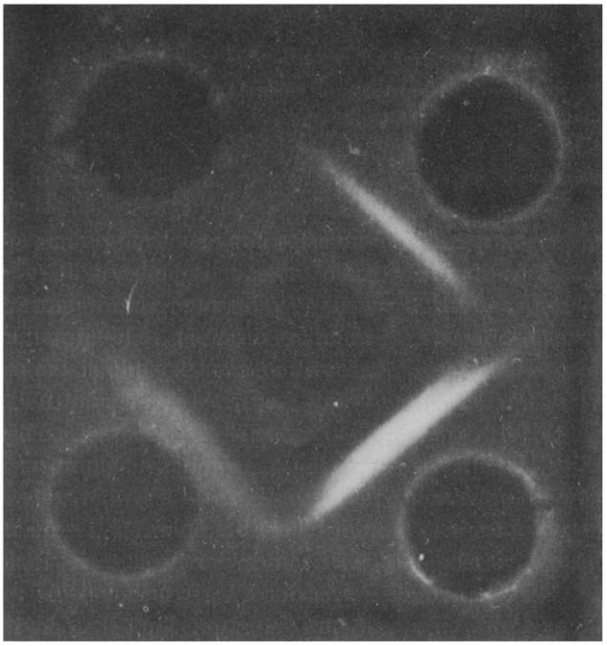
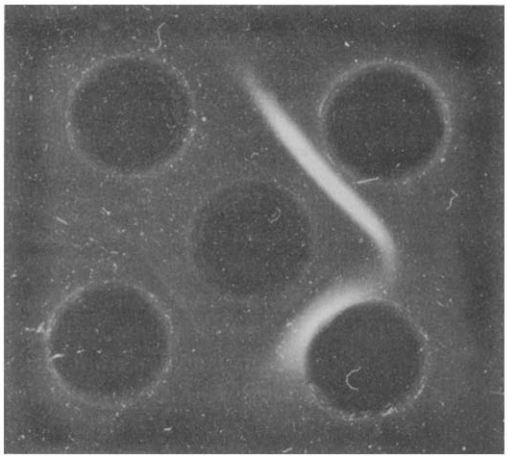



Fig. 1. Photograph (1A) and line-tracing (1B) of double diffusion plates of *bi*A-1 and *bi*A-1 *apl*$^{r}$-7 grown under inducing and non-inducing conditions. The central well contains anti-wild type antiserum. Growth and extraction of mycelia as in table 1. Incubation and staining of plates as described in the text. Contents of each well are indicated in the line drawing and correspond to those in the photograph. *bi*A-1 *apl*$^{r}$-7 is abbreviated to *apl*$^{r}$-7 in the line drawing.

## 3. Results

Table 1 illustrates the typical enzyme activities of *bi*A-1 and *bi*A-1 *apl*$^{r}$-7 in extracts of induced and non-induced mycelia.

Fig. 1A shows a photograph of the xanthine dehydrogenase precipitin lines of these four extracts formed with anti-wild type antiserum and stained specifically for xanthine dehydrogenase activity. It can be seen that the line present in the non-induced *bi*A-1 *apl*$^{r}$-7 is different from the line present in the induced wild type. With extracts of induced *bi*A-1 *apl*$^{r}$-7 both lines are visible. No xanthine dehydrogenase specifically stained lines are visible in the case of non-induced wild type. Fig. 2A shows the same distinction using anti-



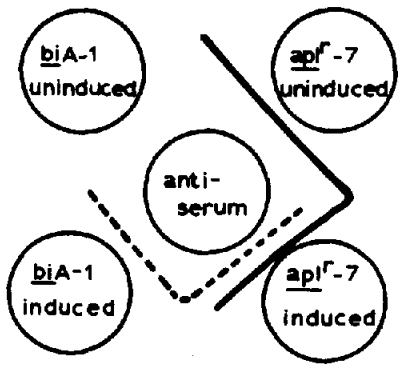



Fig. 2. Photograph (2A) and line tracing (2B) of double diffusion plates of same extracts as in fig. 1 but central well containing anti-*apl*$^{r}$-7 antiserum. The wells are labelled as in fig. 1.

Table 1
Xanthine dehydrogenase activities of *bi*A-1 (wild type) and *bi*A-1 *apl*$^{r}$-7 mycelia grown for 20 hr in minimal medium [2] with 5 mM urea as nitrogen source, and induction after 15 hr growth with 50 μg/ml uric acid. Mycelia were extracted with 10 times v/w 0.1 M pH 8.5 sodium pyrophosphate buffer [2]. Xanthine dehydrogenase activity was measured by following the reduction of cytochrome *c* in the presence of benzyl viologen with hypoxanthine as substrate at 550 nm [4]. Enzyme activities are expressed in percent of the induced wild type. Two independent experiments are shown.

| Strain | Induction | Xanthine dehydrogenase activities | |
|---|---|---|---|
| | | Experiment 1 | Experiment 2 |
| *bi*A-1 | – | 12 | 12 |
| *bi*A-1 | Uric acid | 100 | 100 |
| *bi*A-1 *apl*$^{r}$-7 | – | 125 | 102 |
| *bi*A-1 *apl*$^{r}$-7 | Uric acid | 194 | 144 |

*apl*$^{r}$ antiserum. A strong line is evident opposite the wells containing extracts of *bi*A-1 *apl*$^{r}$-7. A line staining faintly, but clearly visible on the plates is opposite the wells containing induced wild type and induced *bi*A-1 *apl*$^{r}$-7. No lines are visible in the non-induced wild type.

Figs. 1B and 2B show line drawings of the same double diffusion plates. The single lines obtained with extracts of non-induced *bi*A-1 *apl*$^{r}$-7 and induced wild type are non-concurrent and indicate that an immunological difference exists between the xanthine dehydrogenase of these strains.

Results to be presented in detail elsewhere show that mutants allelic to *apl*$^{r}$-7 show similar behaviour in double diffusion plates, while *oxp*$^{r}$ mutants, also constitutive for xanthine dehydrogenase, show only the wild type precipitin line.

Scazzocchio [6] has shown that *apl*$^{r}$-7, constitutive for xanthine dehydrogenase activity when grown on most nitrogen sources, is inducible when grown on nitrate. Analysis by agar diffusion of extracts of mycelium grown in the presence of nitrate indicates that nitrate acts by eliminating specifically the constitutive *apl*$^{r}$-7 activity. When an *apl*$^{r}$-7 mutant is induced by uric acid in the presence of nitrate, only the wild type precipitin line appears in double diffusion studies. The mechanism of this effect is under investigation.

## 4. Discussion

Xanthine dehydrogenase from uric acid-induced wild type and a non-induced constitutive mutant are antigenically different. The observation that antisera prepared against each of the strains precipitates the enzyme from the other as well, suggests that these enzymes may have a common set of antigenic determinants. It has been found that mutation at the *hx*B locus [1, 3] in linkage group VII eliminates both induced and constitutive activities while mutation at the *hx*A locus [1, 3] in linkage group VI eliminates only the induced activity. *apl*$^{r}$-7 was found to be semi-dominant and overinducible [1]. These observations are consistent with the results presented here, that suggest strongly that *apl*$^{r}$-7 produces an enzyme different from the induced wild type but with a peptide common to the latter.

The significance of the partial identity of the two enzymes to the mechanism of the control of xanthine dehydrogenase in *Aspergillus nidulans* will be examined in another publication.

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