SHORT COMMUNICATION

An Immunological Study of the *Neurospora am* Mutants using Antibodies Prepared against Glutamate Dehydrogenase Subunits

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The enzyme studied in this investigation is L-glutamate NADP⁺ oxidoreductase (deaminating), EC. 1.4.1.4, trivially known as glutamate dehydrogenase (GDH) from *Neurospora crassa*.

The amination deficient mutants (*am* mutants) lack normal GDH activity and fall into two classes, those which possess a protein which reacts with antibodies prepared against GDH (CRM⁺ mutants) and those which possess no such protein (CRM⁻ mutants) (Roberts & Pateman, 1964).

In a previous paper (Roberts, 1969) it was shown that antibodies prepared against the subunits of GDH while inhibiting GDH activity did so by combining at sites other than those at which antibodies against the whole molecule combined. The CRM⁻ nature of some of the *am* mutants might be due to the inability of the mutant polypeptide chains to aggregate to form the whole molecule and be recognized by antibodies directed against the whole protein. All mutants were analysed with antibodies prepared against the subunits for the presence in crude extracts of polypeptide chains possessing antigenic determinants recognized by antibodies prepared against the wild-type subunits.

The mutant *am*-14 was studied in greater detail than the other mutants because of its ability, although CRM⁻, to complement many other mutants (Sundaram & Fincham, 1968).

The subunits were prepared by dialysing GDH against 6M-urea giving 6M-urea GDH and against 0.2% sodium dodecyl sulphate giving dodecyl-SO₄GDH. All other techniques have been described previously (Roberts, 1969). Extracts of some mutants reduced the inhibition of GDH by antibodies against the subunits while extracts of other mutants did not. Because of the possibility of mutant subunits being attacked by proteolytic enzymes when extracted from the mycelium, extracts were made in buffer containing 1.5 mg./ml. bovine serum albumin. In no case did the presence of bovine serum albumin in the buffer alter the result of a CRM test.

A summary of the CRM data for *am* mutants as detected by anti-enzyme studies both from this work and from earlier work (Roberts & Pateman, 1964) is given in Table 1.

Analysis on double diffusion plates shows that all the mutants identified as being CRM⁺ with antibodies against GDH are CRM⁺ with antibodies against the subunits and possess both conformers of GDH (Roberts, 1969), while mutants previously detected as being CRM⁻ are CRM⁻ with antibodies against the subunits.

In a similar study on alkaline phosphatase from *Escherichia coli* Schlesinger (1967) detected mutants which failed to produce material which reacted with antibodies prepared against

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the whole protein, but did produce material which reacted with antibodies prepared against subunits. This result he interpreted as being due to mis-sense mutations which formed polypeptide chains unable to aggregate to form the whole protein. No *am* mutants of this type were detected.

The CRM⁻ *am* mutants must either be deletions or nonsense mutations which produce only a small part or no part of the polypeptide chain; or if mis-sense mutations must be mutations which result in a polypeptide chain which is either unrecognized by any of the antibodies tested or else the polypeptide is so unstable as to be broken down as soon as it is synthesized. Such breakdown was not prevented by the addition of carrier protein to the extracts nor was a very small amount of unstable product detected by using large amounts of the material in the tests.

Table 1. *Immunological analysis of the am mutants*

Mutant	CRM expressed as % GDH in a crude extract of wild-type. Analysed with anti-GDH	CRM analysed with anti-6M-urea GDH	CRM analysed with anti-dodecyl-SO ₄ GDH	Precipitin bands on double diffusion plates Analysed with:			
				Anti-GDH	Anti-6M-urea GDH	Anti-dodecyl-SO ₄ GDH	Anti-6M-urea GDH. Antigens in 6M-urea
Wild	100	+	+	1	2	2	2
<i>am-1</i>	78	+	+	1	2	2	2
<i>am-2</i>	79	+	+	1	2	2	2
<i>am-3</i>	94	+	+	1	2	2	2
<i>am-4</i>	56	—	—	1	2	2	2
<i>am-5</i>	3	—	—	0	0	0	0
<i>am-6</i>	8	—	—	0	0	0	0
<i>am-7</i>	72	—	—	1	2	2	2
<i>am-8</i>	5	—	—	0	0	0	0
<i>am-9</i>	0	—	—	0	0	0	0
<i>am-10</i>	+	+	+	1	2	2	2
<i>am-11</i>	6	—	—	0	0	0	0
<i>am-13</i>	88	—	—	1	2	2	2
<i>am-14</i>	9	0	0	0	0	0	0
<i>am-15</i>	5	0	0	0	0	0	0
<i>am-16</i>	0	0	0	0	0	0	0
<i>am-17</i>	2	0	0	0	0	0	0
<i>am-18</i>	0	0	0	0	0	0	0
<i>am-19</i>	59	+	+	1	2	2	2

— = not tested.

Sundaram & Fincham (1968) have already invoked the unstable polypeptide chain hypothesis for the mutant *am-14* which although CRM⁻ by all criteria complements well with a number of mutants and must thus produce some product of the mutant *am* gene.

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