NEW MUTATIONAL VARIANTS OF NEUROSPORA NADP-SPECIFIC GLUTAMATE DEHYDROGENASE[1]

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ABSTRACT

The *am* locus of Neurospora codes for NADP-dependent glutamate dehydrogenase (GDH). Four new *am* mutants that produced mutationally altered GDH have been characterized. Mutant am_{119} is a CRM-negative, complementing mutant that maps between am_2 and am_1. The other three mutants are CRM formers that produce varieties of GDH that can be activated by glutamate or succinate. The GDH of am_{130} and am_{131} is similar in terms of activation properties to that of am_3. The GDH of am_{122} requires very high concentrations of dicarboxylate for activity. The mutation in am_{130} maps between am_{14} and am_2 and resulted in a replacement at residue 75 of the GDH (pro → ser). The mutation in am_{122} maps near am_{11} and apparently resulted in the replacement of the tryptophan residue at position 389 with an unknown amino acid. The mutation in am_{131} maps between am_2 and am_1.

THE *am* gene of Neurospora codes for the enzyme, NADP-dependent glutamate dehydrogenase (GDH) (FINCHAM 1951). The enzyme is a hexamer composed of identical monomers that are 452 amino acid residues long. GDH has allosteric properties that are altered in several *am* mutants (WEST *et al.* 1967; ASHBY, WOOTTON and FINCHAM 1974). By the identification of the amino acid replacements (BRETT *et al.* 1976), it has been possible to determine that at least two regions of the GDH sequence affect allosteric properties. One region is defined by am_2 and am_{19} (altering amino acid residues 141 and 142) and a second region by am_3 and the revertant strain am_{19} R24 (altering amino acid residues 391 and 393, respectively).

Among a new group of *am* mutants isolated by a positive selection procedure (KINSEY 1977) after ultraviolet or nitrous acid mutagenesis, five new mutants that showed intragenic complementation were found (KINSEY and HUNG, unpublished). Two of these, am_{119} and am_{126}, produced no cross-reacting material (CRM). Of these two, am_{126} is a genetically unstable allele that resembles am_{14} somewhat in its complementation properties (KINSEY and FINCHAM, in press). The second mutant, am_{119}, will be briefly discussed below.

The other three complementing mutants, am_{122}, am_{130} and am_{131}, are all

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CRM-positive and produce a form of GDH that can be activated to some extent by substrate. In this communication, we report on their genetic position, the nature of the change in the enzyme in each case, the nature of the amino acid replacement in am_{130} and the probable posiiton of the replacement in am_{122}.

MATERIALS AND METHODS

Neurospora strains: The *am* mutants am_1 through am_{19} and the two wild types, 74A and ORSa, were from the authors' stock collections. Mutants am_{119} through am_{144} were isolated by KINSEY and HUNG (unpublished). All of the stocks used for the complementation tests, CRM determinations, or for the preparation of GDH were reisolations after at least 3 generations of crossing to the wild-type strain 74A. Stocks that were used for mapping carried the markers *cot-1* (allele C102t), *rec-3*, and either *his-1* (allele K83) or *ure-2* (allele 47), which were introduced through a series of crosses. These stocks were of mixed genetic background.

Technique for making and analysing crosses: Crosses were made by simultaneous inoculation of both parents onto plates of WESTERGAARD and MITCHEL's (1947) crossing medium (WM) with an 8.57 cm disc of Whatmans no. 1 filter paper substituted for the normal 2% sucrose. Plates were incubated, inverted, at 25° for at least 3 weeks. Spores were harvested from the lids of plates, using 0.13% sodium hypochlorite to kill residual conidia. The spores were sedimented by centrifugation and resuspended in 0.1% agar water solution. The spore concentration was determined by haemocytometer count. The spores were heat shocked in 0.5% agar (8.3×10^4 spores/ml) at 60° for 45 min. Three ml aliquot portions of the heat-shocked spore suspensions were plated over plates of VOGEL's medium N (VOGEL 1956) containing 1.5% sorbose, 0.2% glucose and 0.2% glycerol (VSGG) and supplemented with glycine (0.02 M) and histidine (50 μg/ml). Only *am* prototrophs will grow on this medium. Three ml aliquot portions of an appropriately diluted spore suspension were overlayered on plates of VSGG supplemented with histidine and alanine (50 μg/ml of each). This medium allowed all viable spores to grow.

Colonies that developed on the VSGG, histidine, glycine plates after 48 hr at 33° were counted. In order to determine the flanking marker status, small bits of the colonies were picked to VSGG and VSGG + histidine plates, as well as to a tube of W.M. containing 2% sucrose and supplemented with histidine. Colonies that grew on VSGG + his, but not on VSGG alone, were mutant at the *his-1* locus. The cultures growing on the tube of WM + histidine were tested for their constitution at the *ure-2* locus by placing a piece of filter paper saturated with a filter-sterilized solution of 6% urea containing 0.4% phenolred on the culture surface. Cultures that produced urease turned the filter paper red.

Determination of cross-reacting material (CRM): CRM was determined by both the enzyme protection assay technique of ROBERTS and PATEMAN (1964) as modified by KINSEY (1977) and the rocket immunoelectrophoresis technique of LAURELL (1966). Antibody was prepared in rabbits against purified GDH by A. TAIT.

GDH extraction, assay and purification: Crude extracts of mycelium grown in 50 ml liquid standing cultures were made in 0.05 M sodium phosphate, 1.0 mM EDTA, pH 7.4, either by grinding with sand in a chilled mortar (10 parts of buffer to 1 of damp mycelium) or by stirring an equivalent amount of powdered lyophilized mycelium into the buffer. Extracts were clarified by centrifugation and contained about 5 mg protein/ml. Enzyme activity was assayed as previously described (CODDINGTON, FINCHAM and SUNDARAM 1966) at 35°. Large batches of mycelium were grown, and the enzyme was purified essentially as described by ASHBY, WOOTTON and FINCHAM (1974), but the final step of gradient elution from a column of DEAE-Sephadex (Pharmacia A50) was omitted; fractional precipitation of the Sephadex G200 fractions with 40% saturated ammonium sulphate was generally adequate for purification to the point where only faint contaminant bands were visible on polyacrylamide gels at high loadings.

For the enzyme kinetic studies (Figures 2, 3, 4), crude extracts were fractionated with ammonium sulphate, and the protein precipitating between 40 and 55% saturation was taken up in 0.5 M sodium phosphate, 0.001 M EDTA buffer, pH 7.0, and dialyzed thoroughly against the same

buffer. This treatment gives an approximately 4-fold purification over crude extract and eliminates glutamate-independent NADP reduction in the assay.

Tryptic peptide isolation, amino acid analysis and sequencing: Carboxy-methylated GDH was digested with trypsin, and the tryptic peptides were fractionated on a Dowex AG50 × 4 column, as described by WOOTTON *et al.* (1975). Appropriate fractions were pooled, dried and refractionated on a column of Biogel P4 and sometimes also on Sephadex G25, equilibrated with 0.05 M ammonium bicarbonate in each case (WOOTTON *et al.* 1975). Fractions from columns were monitored both by absorbancy at 280 nm and (in the case of the P4 and G25 columns) at 220 nm, and by chromatography on TLC (silica gel) plastic sheets (E. Merck, Darmstadt). After development with isopropanol-acetic acid-water (4:1:1 by vol), peptides of interest were tentatively identified by various staining techniques (WOOTTON *et al.* 1975). About one-tenth of the purified peptide was taken for amino acid analysis with a Rank-Hilger "Chromaspek" analyzer. Half of the remainder was usually enough for amino acid sequencing by the Dansyl-Edman method as modified by WOOTTEN *et al.* (1975).

RESULTS

Mapping analysis: Crosses of the general constitution *ure-2* am_x; *rec-3*; *cot-1* × am_y *his-1*; *rec-3*; *cot-1* were made. Prototrophic am^+ recombinants were counted and the flanking marker constitution of the prototrophs was determined as indicated in MATERIALS AND METHODS. In a few cases, crosses of the general constitution am_x; *rec-3*; *cot-1* × am_y *his-1*; *rec-3*; *cot-1* were analyzed for prototrophic recombinant frequency only. The results of both kinds of crosses are shown in Table 1. Two kinds of control experiments were performed that are not listed in the table. First, all *am* mutants used were crossed to themselves in opposite mating type and at least 5×10^6 viable spores analyzed. No prototrophic revertants were found for any of these mutants. Second, the flanking marker *ure-2*, which had not been previously used for fine-structure analysis at the *am* locus, was tested in a set of crosses involving *am* alleles not shown here to determine the effect of *ure-2* on viability of the prototrophic progeny. No effect was found. The order was deduced on the basis of the inequality of the two *parental* combinations of flanking markers—the "polarity of conversion" criterion (SMYTH 1973; FINCHAM 1967, 1974). The order is given according to SMYTH's convention, which places am_6 centromere-proximal and am_9 distal. In these crosses, sites were ordered by the polarity criterion only if differences were significant at the 5% probability level, except that the order $am_2 \rightarrow am_{131}$ was based on data with a probability level of 10%.

On the basis of conversion polarity, am_{122} can be placed distal to am_2 (amino acid residue 141). Because of strong complementation with am_1, which leads to the formation of large numbers of pseudo-wild-type ascospores, it cannot be accurately ordered with respect to am_1, and the recombination frequency given for this cross is rather uncertain. On the basis of its very tight linkage to am_{11}, which is known to be distal to am_1 (residue 336), we presume that am_{122} is distal to am_1. This idea is supported by our preliminary assignment of the lesion in am_{122} to a residue in peptide T32 (see below).

Conversion polarity unambiguously places am_{130} between am_{14} and am_2 (residues 20 and 141); to judge from the recombination frequencies, it is likely

TABLE 1

Frequencies of and distribution of flanking markers among am *prototrophic recombinants from crosses of the general constitution* + am_x his-1 × ure-2 am_y

+ am_x his-1	ure-2 am_y +	Frequency of am^+ progeny per 10^5 live spores	Distribution of flanking markers of the am^+progeny +h	u+	uh	++	Total	Deduced order
130	14	1.4	6	42	9	9	66	14 → 130
130	2	7.27	34	11	9	2	56	130 → 2
130	9	30.0	59	9	1	7	76	130 → 9
135	14	5.0	3	32	6	7	48	14 → 135
135	2	5.51	11	33	6	10	60	2 → 135
135	1	24.37	79	6	3	2	90	135 → 1
144	14	8.4	8	74	12	12	105	14 → 144
144	2	4.17	3	33	1	4	40	2 → 144
144	1	9.24	20	0	5	5	30	144 → 1
131	2	3.02	5	12	0	1	18	2 → 131
131	1	~ 4	strong complementation					?
135	131*	0.9	—	—	—	—	—	?
144	131*	0.07	—	—	—	—	—	?
122	14	49.0	4	60	5	4	73	14 → 122
122	2	35.4	22	54	8	8	92	2 → 122
122	1	~ 4	strong complementation					?
122	11	0.13	1	0	1	0	2	?
122	14	10	2	60	6	12	80	14 → 119
119	2	2.22	7	12	0	10	29	?
119	1	~ 3	recombinants not analyzed					?
131	119*	0.89	—	—	—	—	—	?

* These stocks contained a wild-type allele at the *ure-2* locus.
Flanking marker distribution was not determined.

to be closer to the former. This position has been corroborated by amino acid sequence analysis (see below).

The positions of am_{131} and am_{119} have been less accurately determined; however, the positions of am_{135} and am_{144}, two other CRM-forming mutants, have been determined unambiguously as being between am_2 and am_1. On the basis of recombinant frequencies, am_{131} can be shown to map close to am_{135} and am_{144}; thus, it appears that the position of am_{131} is between am_2 and am_1. Likewise, am_{119} maps close to am_{131} so that its position is also apparently between am_2 and am_1. Figure 1 shows the present state of the genetic map.

Complementation: A complementation matrix that includes am_{119}, am_{122}, am_{130} and am_{131}, as well as all previously known complementing *am* mutants, has recently been published (KINSEY and FINCHAM, in press). Figure 2 shows the complementation relationships of these mutants in the form of a circular map.

Cross-reacting material: Crude extracts from am_{119}, am_{122}, am_{130} and am_{131} were assayed for CRM using both the enzyme protection assay (ROBERTS and PATEMAN 1964) and rocket immunoelectrophoresis (RI) (LAURELL 1966). The extracts of am_{119} showed no CRM in either test. The extracts of am_{122}, am_{130} and am_{131}, on the other hand, all showed CRM by both techniques. Table 2 compares

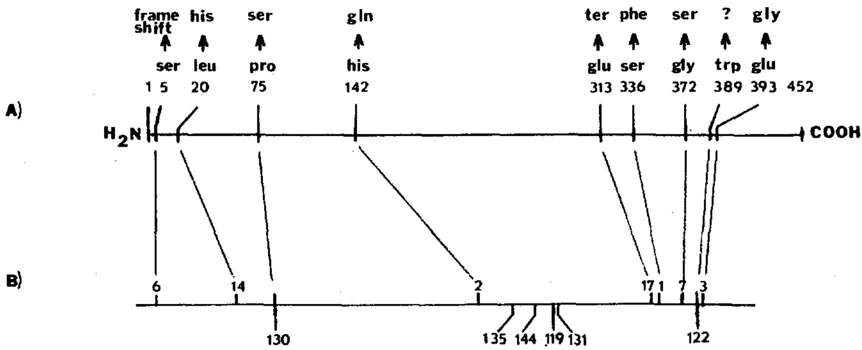

FIGURE 1.—The present state of the genetic map of the Neurospora *am* gene. (A) The polypeptide chain with the amino acid replacements that have been identified (see BRETT *et al* 1976; FINCHAM and BARON 1977; SEALE *et al.* 1977; this report). The mutation to a one-base addition frameshift in the serine 5 codon was shown by SIDDIG, KINSEY, FINCHAM and KEIGHREN (in press). The symbol *ter* refers to chain termination. (B) The genetic map of the *am* locus based upon the data of SMYTH (1973) and data from this paper. The relative positions of the mutations shown above the line are based upon SMYTH's data; those shown below the line are based on data in this paper. The relative order of mutations below the line with respect to those above the line is unambiguous. The relative order of the cluster of mutants 135, 144, 119 and 131 is only tentative..

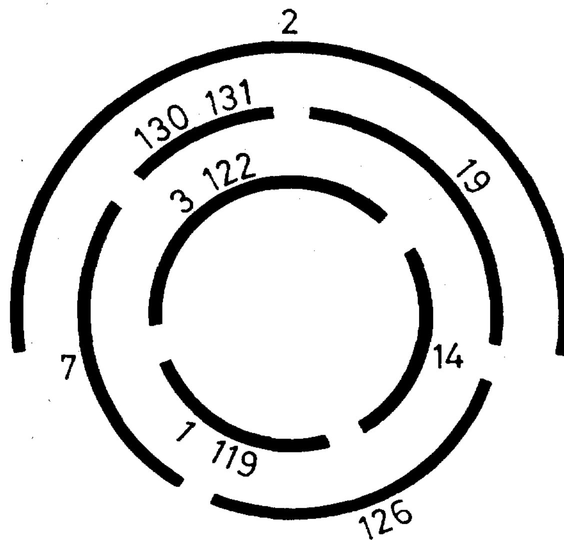

FIGURE 2.—Complementation map of *am*. This is the map obtained using the smallest repeatable complementation responses. The complementation between the mutant pairs 19 + 130, 19 + 131, 19 + 122, 19 + 3, 7 + 130, 7 + 131 and 2 + 14 was very weak (data from KINSEY and FINCHAM 1979).

TABLE 2

Cross reacting material produced by am *mutants*

Extract	mg Protein per ml	Rocket height (mm) 3 μl of extract	Average rocket height (mm) per mg protein	GDH content as % of wild type†
ORSa (WT)	25.2	7.06 0.4 (5)*	93.4	100
122	30.8	10.16 0.3 (5)	109.9	118
130	21.0	6.72 0.3 (5)	106.7	114
131	33.6	11.45 1.2 (4)	113.6	122

* Number in parentheses is the number of replicate determinations.
† Rocket height is nearly proportional to amount of GDH protein.

crude extracts of wild type (ORSa), am_{122}, am_{130} and am_{131} (stationary cultures grown on minimal medium containing 0.5 M L-glutamate for 48 hr at 25°) with respect to CRM production as measured by rocket immunoelectrophoresis. The average rocket height in each case was normalized on the basis of protein concentration to give a specific "GDH" content. The values for the three mutants were then normalized to that of the wild type, ORSa. Clearly, all three of the mutants produce at least as much "GDH" protein as does wild type; in fact, they produce slightly more "GDH" protein by this criterion. This is consistent with past observations we have made that many *am* mutants yield more purified GDH protein than does the wild type grown under identical conditions.

Properties of mutant enzymes: Extracts of the three mutants (am_{122}, am_{130} and am_{131}), which were both complementing and CRM-positive, all showed activity of GDH when assayed in system "C" (reduction of NADP in presence of 0.15 M L-glutamate at pH 8.5; CODDINGTON, FINCHAM and SUNDARAM 1966), especially when the enzyme sample was preincubated with the Tris-glutamate mixture for two minutes before addition of the NADP. Specific activities found in one experiment are shown in Table 3. None of the mutant extracts was active in the glutamate-synthesizing assay system (2-oxoglutarate, NH4+ and NADPH), which shows high activity for the wild-type enzyme.

The mutant enzyme varieties formed by am_{122}, am_{130} and am_{131} resembled

TABLE 3

*Specific GDH activities measured in assay system "C"**

	Crude extracts	Purified enzyme
Wild type	760	120,000
am_2	1010	——
am_3	870	——
am_{122}	110	26,000
am_{130}	270	77,500
am_{131}	1000	95,000

* Final concentrations: 0.15 M L-glutamate, 0.10 M Tris, pH 8.5, 1.7×10^{-5} M NADP, 35°; enzyme sample incubated for 2 min with the Tris-glutamate (2.8 ml) before addition of the NADP (0.2 ml).

the previously investigated am_2 and am_3 mutant varieties (FINCHAM 1962) in being activated by a combination of high pH and succinate—treatment of the same kind as that required for the allosteric activation of the wild-type enzyme, but more extreme (WEST *et al.* 1967; ASHBY, WOOTTON and FINCHAM 1974). Figure 3 shows the results of experiments on the activation requirements of the different GDH varieties. Dialyzed ammonium sulphate fractions of cell extracts were incubated for 10 minutes at 35° in different concentrations of succinate at a number of different pH values before being assayed with a relatively low concentration of glutamate so as to minimize further activation in the assay system. The am_{131} enzyme behaved very much like that from am_3, except that it was more easily activated. This mutant GDH seems to have almost the minimum abnormality consistent with loss of *in vivo* function and an auxotrophic phenotype. In its activation requirements, the am_{130} enzyme was close to that of am_3, but differed from the latter variety in a feature not shown in the figure, a very rapid loss of activity in the assay system. The rate of reduction of NADP fell nearly to zero within two minutes, long before the equilibrium position of the reaction had been reached. This loss of activity was also apparent when the higher glutamate concentration of 0.15 M (System "C") was used, and it seems likely that the factor responsible was not the inadequate concentration of positive allosteric effector molecules, but rather the formation of NADPH, which is a negative effector for the wild-type enzyme under some conditions

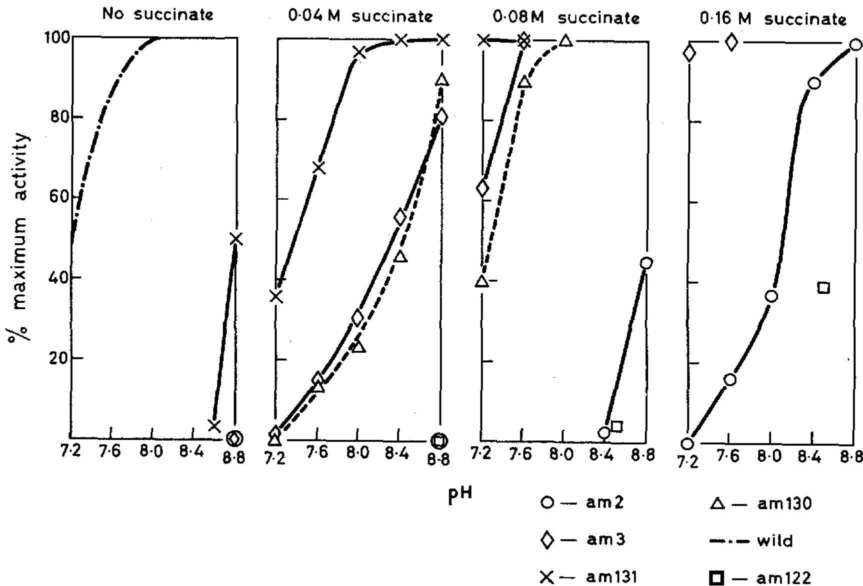

FIGURE 3.—Conditions for activation of various mutant forms of GDH. Assays for activity were made with a recording spectrophotometer in a system containing 33.3 mM sodium L-glutamate and 1.7×10^{-5} M NADP in 0.1 M Tris HCl at pH 8.5 and 35°. The enzyme was added as the final addition after 10 min preincubation at 35° in 0.1 M Tris at the pH values and with the succinate concentrations indicated. Initial rates of NADP reduction were measured.

(WEST *et al.* 1967). A similar (though less extreme) effect is seen with the am_2 enzyme, and it seems likely that these two mutant enzyme varieties are hypersensitive to the negative allosteric effect of NADPH.

Even under the most activating conditions shown in Figure 3, am_{122} had low activity compared with wild type or the other mutants. When the GDH activity of am_{122} was assayed with increasing glutamate concentrations in the presence or absence of succinate (Figure 4), it became obvious that it required a uniquely high concentration of effector. Succinate at a concentration adequate for full activation was a potent competitive inhibitor with respect to glutamate. The potential maximum activity of am_{122} is approached only when glutamate, at very high concentrations, acts as both substrate and activator. The activity of the am_{122} enzyme requires the presence of high dicarboxylate concentration in the reaction mix and is not maintained for a significant time after the removal of the dicarboxylate. Even under the most activating conditions, there was no activity in the glutamate synthesis assay.

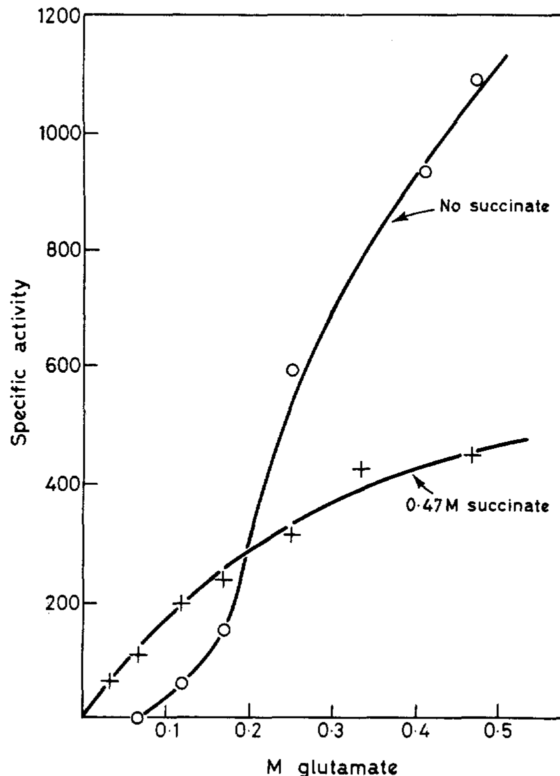

FIGURE 4.—Activation of am^{122} GDH by glutamate and succinate. Assay conditions as in legend to Figure 3 except that the pH 8.5 was used throughout, the glutamate concentration was varied and the reaction was started by addition of the NADP in 0.2 ml following 2 min preincubation of the enzyme at 35° in the remainder of the reaction mixture (2.8 ml). Specific activity, based on initial rate, is expressed as $100 \times \Delta OD_{340}$/min/mg protein.

Figure 5 shows a Michaelis-Menten plot for the wild type and am_{122} GDH in the presence and absence of succinate. The K_m (in the absence of succinate) for wild-type GDH is *ca.* 30 mM. The apparent K_m's in the presence of 0.24 M and 0.48 M succinate are 250 mM and 650 mM, respectively. From these values, it is possible to calculate a K_i for succinate. Values of 23 mM and 33 mM were obtained from the 0.48 M and 0.24 M succinate data, respectively, which are not very different from the K_m for glutamate. Although an accurate K_m for glutamate in am_{122} GDH cannot be calculated from these data, the apparent K_m's for glutamate in the presence of 0.24 M and 0.48 M succinate are the same as those calculated for wild type at the same succinate concentrations. It also appears that the V_{max} for am_{122} GDH is similar to that of wild-type GDH.

Peptide profile, isolation and sequencing: The map position of the mutant am_{130} suggested the possibility of an amino acid replacement in tryptic peptide T8. This peptide was isolated from a tryptic digest of purified am_{130} GDH, and amino acid analysis showed an apparent loss of proline and two instead of one serine residues, as compared with the wild type. This was confirmed by complete sequence determination of the peptide, which showed that the proline present at residue 75 in the wild-type sequence had been replaced by serine (Table 4).

An amino acid replacement in the GDH variety produced by the mutant am_{122} has not yet been fully identified, but it seems likely that the residue affected is in tryptic peptide T32, which contains tryptophan and is easy to identify in the wild type. A search of the tryptic peptide profile of purified am_{122} GDH revealed no tryptophan-containing peptide in the usual T32 position and no unusual tryptophan-containing peptide anywhere else. J. C. WOOTON (personal communication) has confirmed this finding and has obtained spectroscopic evidence that the purified am_{122} GDH has about one residue less of tryptophan per monomer than wild-type GDH. Even though it has not yet been possible to identify an altered T32 peptide in am_{122}, it seems very likely that Trp-389 has been replaced. If it has been substituted by arginine, which is one possibility, the

TABLE 4

The amino acid replacement in tryptic peptide T8 from am$_{130}$

	Amino acid analysis (yields relative to Phe = 1.0)														
	Asx	Thr	Ser	Glx	Pro	Gly	Ala	Val	Ile	Leu	Tyr	Phe	His	Lys	Arg
am_{130} T8	1.7	0.3	1.9	0.95	0.3	1.4	1.4	0.9	0.1	1.0	0.8	1.0	0.2	1.2	0.3
Likely no. of residues	1	–	2	1	–	1	1	1	–	1	1	1	–	1	–
cf. Wild type T8*	1	–	1	1	1	1	1	1	–	1	1	1	–	1	–

	Amino acid sequence (— indicates positive identification of the end-group after the Edman round)
am_{130} T8	Val – Glx – Phe – Asx – Ser – Ala – Leu – Gly – Ser – Tyr (Lys)
cf. Wild type*	Val – Glu – Phe – Asp – Ser – Ala – Leu – Gly – Pro – Tyr – Lys
Residue no.	67 70 75

* From WOOTTON *et al.*, 1975.

sequence corresponding to T32 in am_{122} would be split by trypsin to give two new peptides.

No progress has yet been made on the identification of the presumed amino acid replacement in am_{131}; it may be that the residue affected is in one of the insoluble peptides T20 and T23, which have not yet been recovered from the mutant protein.

DISCUSSION

We have characterized four new *am* mutants that produce variants of GDH. The first mutant, am_{119}, maps between am_2 (corresponding to amino acid residue 141) and am_1 (corresponding to amino acid residue 336). We can be sure that it produces a variant of GDH on the basis of its ability to complement, even though we have been unable to detect any GDH or CRM in extracts of this mutant. This behavior of am_{119} is somewhat anomalous in the sense that it behaves in complementation exactly as does am_1, which can contribute a stable and conformationally nearly normal (though inactive) monomer to a mixed oligomer (CODDINGTON, FINCHAM and SUNDARAM 1966; WATSON and WOOTON 1978). It may seem difficult to attribute a similar role to am_{119} when we find it to produce no GDM or CRM. The most likely possibility is that the am_{119} monomer has normal tertiary conformation, but very low quarternary stability.

The other three mutants all complement and produce both CRM and enzyme that can be activated by succinate or glutamate. Mutation am_{130} was located between am_{14} and am_2 by mapping, and the amino acid replacement has been shown to be at residue 75 (pro → ser), which is in agreement with its map position. Mutation am_{122} maps to the left of am_2 close to the position of am_{11}. In apparent confirmation of this position, we find an apparent absence of tryptophan 389, though what replaced it is not yet known. This would be consistent with its map position.

Mutation am_{131} has been indirectly mapped between am_2 and am_1. We have no information with regard to the altered amino acid sequence in this mutant.

With the addition of these mutant GDH varieties to our repertoire, it now seems likely that there are more than two areas of the GDH molecule in which amino acid substitutions affect allosteric properties. Previously, BRETT *et al.* (1976) had postulated that residues 141 and 142 and residues 391 and 393 were important for determining a normal balance between active and inactive configurations. The mutation in am_{122}, if it does result in the replacement of tryptophan 389, would simply represent an extension of a previously postulated region; however, the involvement of residue 75 adds a new region, as presumably does the am_{131} change, which lies somewhere between amino acid residues 141 and 336, perhaps (taking the recombination frequencies of Table 1 at face value) around residue 180–200.

An interesting observation arising from the study of am_{122} protein has been the observation that succinate, which as been known for some time to be an activator of Neurospora GDH (WEST *et al.* 1967), is a competitive inhibitor

with a K_i similar to the K_m for glutamate. It now seems likely that all of the activating effects of succinate are due to binding at the substrate site.

LITERATURE CITED

ASHBY, B., J. C. WOOTTON and J. R. S. FINCHAM, 1974 Slow conformational changes of a *Neurospora* glutamate dehydrogenase studied by protein fluorescence. Biochem. J. **143**: 317–329.

BRETT, M., G. K. CHAMBERS, A. A. HOLDER, J. R. S. FINCHAM and J. C. WOOTTON, 1976 Mutational amino acid replacements in *Neurospora crassa* NADP-specific glutamate dehydrogenase. J. Mol. Biol. **106**: 1–22.

CODDINGTON, A., J. R. S. FINCHAM and T. K. SUNDARAM, 1966 Multiple active varieties of *Neurospora* glutamate dehydrogenase formed by hybridization between two inactive mutant proteins in vivo and in vitro. J. Mol. Biol. **17**: 503–512.

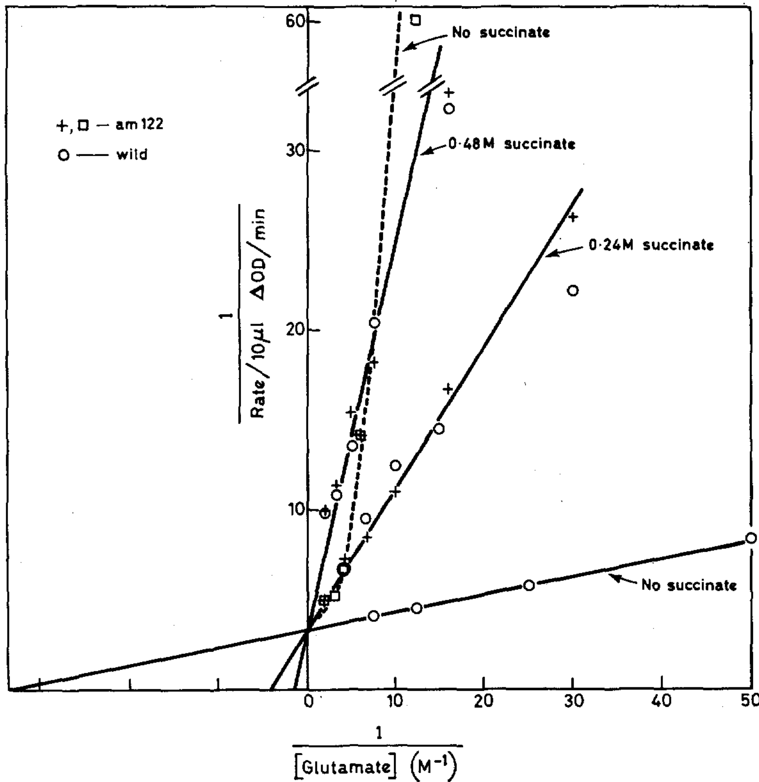

FIGURE 5.—Michaelis constants, maximum activities and the effects of succinate for wild-type and am_{122} GDH. Assays as in legend for Figure 3. Reaction rates are expressed as $\Delta OD_{340}/10$ µl. Protein concentrations were 11 mg/ml for am_{122} and 12 mg/ml for wild type. Maximum specific activities (ΔOD_{340}/mg protein × 100) are calculated as 2,500 for wild-type and 2,700 for am_{122} GDH. Apparent Michaelis constants are approximately 30 mM, 230 mM and 480 mM in the presence of zero, 0.24 M and 0.48 M succinate for both enzymes. The implied K_i with respect to succinate is approximately 30 mM for both enzymes. Symbols used; (○), wildtype (□), am_{122} in the absence of succinate. A broken line is also used to designate this particular curve. (+), am_{122} in the presence of either 0.24 M or 0.48 M succinate.

FINCHAM, J. R. S., 1951 The occurrence of glutamic dehydrogenuase in *Neurospora* and its apparent absence in certain mutant strains. J. Gen. Microbiol. **5**: 793–806. ——, 1962 Genetically determined multiple forms of glutamic dehydrogenase in *Neurospora crassa.* J. Mol. Biol. **4**: 257–274. ——, 1967 Recombination within the *am* gene of *Neurospora crassa.* Genet. Res. **9**: 49–62. ——,1974 Negative interference and the use of flanking markers in fine-structure mapping in fungi. Heredity **33**: 116–121.

FINCHAM, J. R. S. and A. J. BARON, 1977 The molecular basis of an osmotically reparable mutant of *Neurospora crassa* producing unstable glutamate dehydrogenase. J. Mol. Biol. **110**: 627–642.

KINSEY, J. A., 1977 Direct selective procedure for isolating *Neurospora* mutants defective in nicotinamide adenine dinucleotide phosphate-specific glutamate dehydrogenase. J. Bacteriol. **132**: 751–756.

KINSEY, J. A. and J. R. S. FINCHAM, 1979 An unstable allele of the *am* locus of *Neuropsora crassa.* Genetics **93**: 577–586.

LAURELL, C., 1966 Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. Anal. Biochem. **15**: 45–52.

ROBERTS, D. B. and J. A. PATEMAN, 1964 Immunological studies of amination deficient strains of *Neurospora crassa.* J. Gen. Microbiol. **34**: 295–305.

SEALE, T., M. BRETT, A. J. BARON and J. R. S. FINCHAM, 1977 Amino acid replacements resulting from suppression and missense reversion of a chain-terminator mutation in Neurospora. Genetics **86**: 261–274.

SIDDIG, M. A. M., J. A. KINSEY, J. R. S. FINCHAM and M. KEIGHREN, 1980 Frameshift mutations affecting the N-terminal sequence of *Neurospora* NADP-specific Glutamate Dehydrogenase. J. Mol. Biol. **137**: 125–135.

SMYTH, D. R., 1973 A new map of the *amination*-1 locus of *Neurospora crassa,* and the effect of the *recombination*-3 gene. Aust. J. Biol. Sci. **26**: 1355–1370.

VOGEL, H. J., 1956 A convenient growth medium for *Neurospora* (Medium N). Microbiol. Genet. Bull. **13**: 42–43.

WATSON, D. H. and J. C. WOOTTON, 1978 Subunit ratios of separated hybrid hexamers of *Neurospora* NADP-specific glutamate dehydrogenase containing complementing mutationally modified monomers. Biochem. J. **175**: 1125–1133.

WEST, D. J., R. W. TUVESON, R. W. BARRATT and J. R. S. FINCHAM, 1967 Allosteric effects in nicotinamide adenine dinucleotide phosphate-specific glutamate dehydrogenase from *Neurospora.* J. Biol. Chem. **242**: 2134–2138.

WESTERGAARD, M. and H. K. MITCHELL, 1947 Neurospora V. A synthetic medium favouring sexual reproduction. Am. J. Botany **34**: 573–577.

WOOTTON, J. C., J. G. TAYLOR, A. A. JACKSON, G. K. CHAMBERS and J. R. S. FINCHAM, 1975 The amino acid sequence of *Neurospora* NADP-specific glutamate dehydrogenase. Biochem. J. **149**: 739–748.

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