SUBUNIT INTERACTION OF A TEMPERATURE-SENSITIVE ALCOHOL DEHYDROGENASE MUTANT IN MAIZE[1]

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TEMPERATURE-sensitive mutations, i.e., mutations which render enzymes inactive only at certain temperatures, have proven to be extremely useful in genetic analyses. Such mutations are usually detected by the inability of an organism to grow or carry out a particular function at high temperatures. In the course of ethyl methanesulfonate (EMS) mutation studies a temperature-sensitive allele of the alcohol dehydrogenase gene (Adh_1) was obtained in maize. This mutation was recognized as being temperature-sensitive in the course of physical-chemical studies on the enzymes specified by the mutant Adh_1 alleles.

Alcohol dehydrogenase of maize behaves as a dimer. A number of electrophoretically distinguishable mutants have been described and a hybrid isozyme of intermediate mobility is formed in heterozygotes (SCHWARTZ and ENDO 1966). This investigation was initiated to study the interaction of a temperature-sensitive subunit with a wild-type (reference) subunit in heterodimers. Three possible types if interactions are: (a) positive complementation (FINCHAM and PATEMAN 1957), rendering the heterodimer fully active at high temperature; (b) negative complementation (FOLEY, GILES and ROBERTS 1965), resulting in the heterodimer becoming temperature-sensitive and inactive at the high temperature; and (c) noncomplementation, in which the subunits do not interact and each retains its own property in the heterodimer. A c-type heterodimer might be expected to have only half of the wild-type activity at high temperatures if only one of the two subunits is active.

MATERIALS AND METHODS

The mutations were induced by treating kernels of the genotype Adh_1^S/Adh_1^S with EMS according to the procedure of BRIGGS, AMANO and SMITH (1965). The plants grown from the treated kernels were pollinated by homozygous Adh_1^F plants. Mutant sectors on the ears were detected by screening 12 F_1 kernels from each ear electrophoretically for mutations of the Adh_1^S allele. The procedures for starch gel electrophoresis and development of ADH zymograms have been previously described (SCHWARTZ and ENDO 1966). In this study enzyme was extracted from the dry kernels by grinding them in a Wiley Mill through a 20 mesh screen, steeping the meal in 0.005 M sodium phosphate buffer, pH 8.0, for 15 min at a constant w/v ratio (1 gram meal per 3 ml buffer). The slurry was centrifuged at 39,000 $\times$ g for 15 min and the supernatant used as the enzyme extract. Enzyme activity was measured by following the reduction of NAD by optical density measurements at 340 mμ as previously described (EFRON and SCHWARTZ 1968). A unit of activity represents a change of OD of 0.001 per minute.

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The temperature-sensitive allele is designated $Adh_1^{S(1108)}$ and for simplicity the mutant subunit which it specifies will be designated S^m. In this study the $Adh_1^{C(t)}$ allele which specifies a fast migrating isozyme was used as the wild-type gene for comparison with the temperature-sensitive mutant. In order to eliminate differences in enzyme level due to other unrelated genes which influence protein synthesis in general, comparisons were made on pooled F_2 kernels from single ears. $Adh_1^{C(t)}/Adh_1^{S(1108)}$ plants were self-pollinated and extracts were made from the pooled $Adh_1^{C(t)}/Adh_1^{C(t)}$, $Adh_1^{C(t)}/Adh_1^{S(1108)}$, and $Adh_1^{S(1108)}/Adh^{S(1108)}$ kernels. Since the Adh_1 alleles produce no detectable phenotypic differences, genotyping was done by removing a small portion of the embryo of each kernel for electrophoretic analysis prior to pooling and mealing the kernels. The $Adh_1^{C(t)}/Adh_1^{S(1108)}$ heterozygotes are recognized by the presence of a hybrid enzyme.

RESULTS

The $Adh_1^{S(1108)}/Adh_1^{S(1108)}$ kernels have only about 23% of the ADH activity of the wild-type $Adh_1^{C(t)}/Adh_1^{C(t)}$ kernels (Table 1, column a). Since as will be shown in this paper the $Adh_1^{S(1108)}$ mutant is temperature sensitive, the reduction in activity probably represents inactivation of the mutant enzyme *in vivo* during plant growth. As can be seen from the table, the $Adh_1^{C(t)}/Adh_1^{S(1108)}$ kernels have considerably more activity than would be expected if there were no complementation between the C^t and S^m subunits. In fact, from the data one can calculate that even though the S^mS^m dimer has lost over three-quarters of its activity, the C^tS^m heterodimer is as active as the C^tC^t homodimer. If we assume that the $Adh_1^{C(t)}$ and $Adh_1^{S(1108)}$ alleles produce equal numbers of subunits, then the three dimers found in the $Adh_1^{C(t)}/Adh_1^{S(1108)}$ heterozygotes should be in a ratio of $1C^tC^t:2C^tS^m:1S^mS^m$. Thus, one-quarter of the dimers in the heterozygote are C^tC^t and should contribute one-quarter of the activity found in the $Adh_1^{C(t)}/Adh_1^{C(t)}$ homozygotes or 292.5 units/ml (1170/4). One-quarter of the dimers in the heterozygote are S^mS^m and should contribute one-quarter of the activity found in the $Adh_1^{S(1108)}/Adh_1^{S(1108)}$ homozygotes or 67.5 units (270/4). Thus the C^tC^t and S^mS^m homodimers should contribute 360 units to the total activity. If the C^tS^m heterodimer is as active as the C^tC^t homodimer, the hybrid enzyme should contribute 585 units (2×292.5) giving a total activity of 945 units calculated. The observed value for the $Adh_1^{C(t)}/Adh_1^{S(1108)}$ heterozygous kernels was 940. These data indicate that the S^m subunit is fully active in a heterodimer with C^t.

Wild-type maize ADH is quite stable at 54°C. After 15 min incubation at this temperature, the C^tC^t enzyme retains over 99% of its initial activity (Table 1,

TABLE 1

ADH activity in extracts from pooled F_2 kernels (units/ml)

Genotype	Treatment a Control	b Heat	c Dialysis	d Dialysis and heat
*$Adh_1^{C(t)}/Adh_1^{C(t)}$	1170	1160	1010	850
$Adh_1^{C(t)}/Adh_1^{S(1108)}$	940	840	730	290
$Adh_1^{S(1108)}/Adh_1^{S(1108)}$	270	15	10	0

* Used as wild-type (reference) genotype.

column b). The S^mS^m dimer is highly temperature sensitive showing approximately 94% inactivation after 15 min at 54°C. The extract from the $Adh_1^{C(t)}/Adh_1^{S(1108)}$ heterozygotes is only slightly inactivated (about 10%) by the heat treatment and as will be shown this is due to the strong inactivation of the S^mS^m homodimers in the extract, with the C^tS^m heterodimers remaining unaffected. The $Adh_1^{C(t)}/Adh_1^{S(1108)}$ heterozygous kernels should contain 873.7 units of activity/ml if the C^tS^m heterodimer is as stable to heating as the C^tC^t dimer. The observed value is 840, a close fit.

Since the heterodimer composed of a wild-type and a temperature-sensitive protomer is as heat stable as a wild-type homodimer, complementation must be operating such that the temperature-sensitive subunit is rendered stable to heat by association in a dimer with a heat-stable subunit.

Unheated extracts of $Adh_1^{C(t)}/Adh_1^{S(1108)}$ kernels show two isozyme bands in starch gel zymograms, the C^tC^t and C^tS^m hybrid band with the hybrid band more intense than the C^tC^t band as expected (Figure 1). The same pattern appears in zymograms from heated extracts. The position of the C^tS^m heterodimer isozyme band is the same as the C^tS band so no change in net charge occurred in the $S \rightarrow S^m$ change. The S^mS^m isozyme band is not observed even when the electrophoresis is performed in the cold at 4°C. In fact, even in extracts from $Adh_1^{S(1108)}/Adh_1^{S(1108)}$ kernels, one observes only a very faint S^mS^m isozyme band after prolonged staining of the zymograms. The intensity of the band is much less than

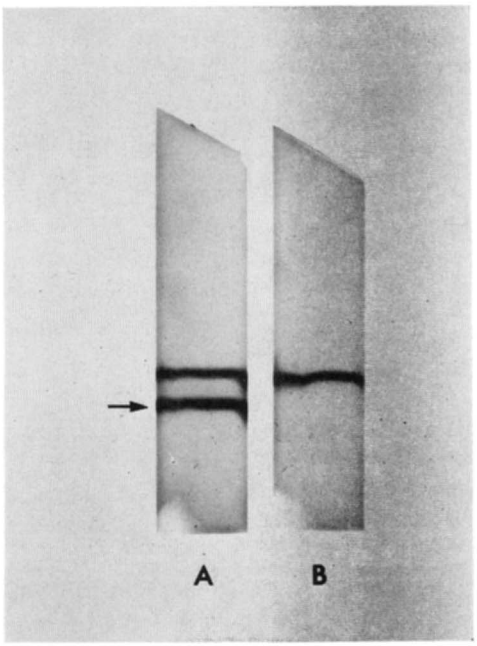

FIGURE 1.—Zymograms of extract from $Adh_1^{C(t)}/Adh_1^{S(1108)}$ kernels. (A) control, (B) dialyzed and heated. The arrow points to the C^tS^m heterodimer. Note that in the dialyzed and heated extract only the C^tC^t isozyme band is seen. The zymograms of extract which were only heated or dialyzed are indistinguishable from the control.

expected on the basis of the relative measured ADH activity in $Adh_1^{C(t)}/Adh_1^{C(t)}$ and $Adh_1^{S(1108)}/Adh_1^{S(1108)}$ kernels. The explanation for this discrepancy became apparent when it was found that in addition to being temperature sensitive, the S^mS^m enzyme is also inactivated by dialysis. During electrophoresis the enzyme is separated from the small molecules in the extract and the S^mS^m isozyme is thereby inactivated.

The C^tC^t enzyme retains over 86% of its activity after overnight dialysis against the sodium phosphate buffer at 4°C, whereas the S^mS^m enzyme has less than 4% of its initial activity (Table 1, column c). Here too it can be shown that the C^tS^m heterodimer has the same stability to dialysis as the wild-type C^tC^t homodimer. The value for the activity of the $Adh_1^{C(t)}/Adh_1^{S(1108)}$ extract after dialysis is calculated to be 760 units/ml if C^tS^m is as stable as C^tC^t, and the observed value is 730 units/ml. Thus the wild-type subunit acts as a dominant conferring both its heat stability and its stability against dialysis to the C^tS^m heterodimer.

The experiments described to this point suggest that the C^tS^m heterodimer is as stable as the wild-type C^tC^t homodimer. However, differences in relative stability are revealed when the dialyzed extract is heated. The extracts were first dialyzed overnight at 4°C then heated for 15 min at 54°C. The data are presented in Table 1, column d. As a result of the combined treatment, the enzyme in the $Adh_1^{S(1108)}$ homozygote is completely inactivated. The wild-type enzyme from the $Adh_1^{C(t)}$ homozygous kernels retains approximately 73% of the initial activity. However, the extracts from the heterozygotes show considerable inactivation, retaining only 31% of the initial activity. Calculations similar to those presented above reveal that the C^tS^m heterodimer is almost 90% inactivated and most of the activity remaining in the $Adh_1^{C(t)}/Adh_1^{S(1108)}$ extract comes from the more stable C^tC^t homodimers. The C^tC^t homodimer in the heterozygous extract contributes 222.5 units of activity/ml to the total (one-quarter of the 850 units/ml measured in the $Adh_1^{C(t)}/Adh_1^{C(t)}$ homozygotes). The S^mS^m dimer is completely inactivated and contributes nothing. The total measured activity is 290 units/ml. This leaves only 67.5 units/ml contributed by the C^tS^m dimer (290 − 222.5). The activity of the C^tS^m dimer in the undialyzed unheated extract was 585 units/ml, thus this heterodimer retains only 11.5% of its activity after the combined treatment, while the wild-type C^tC^t dimer retains 73% of its activity.

Additional proof that it is the C^tS^m heterodimer which is inactivated after the combined dialysis and heat treatments comes from a comparison of the isozyme bands in zymograms of the treated and untreated extracts from the $Adh_1^{C(t)}/Adh_1^{S(1008)}$ kernels (Figure 1). In the treated extract, only the C^tC^t isozyme band is seen; whereas in the control the C^tS^m isozyme band is more intense than the C^tC^t band.

Three possible alternatives were listed in the introduction to this paper for the behavior of heterodimers composed of a wild-type and a temperature-sensitive subunit: positive complementation, negative complementation, or no interaction. The experimental results show positive complementation for heat stability at 54°C and stability after dialysis since the heterodimer acts like the wild-type

homodimer. However, negative complementation seems to be operating in the heterodimer when stability against both dialysis and heat is tested, since the heterodimer more closely resembles the S^mS^m dimer in degree of inactivation. Our working hypothesis to explain these results is that S^m is more sensitive to high temperature and dialysis because the interdimeric bonds between the subunits are weaker or fewer in number. There is sufficient interdimeric bonding strength to maintain the dimer structure in the C^tS^m heterodimer when the enzyme is subjected to heat treatment or to dialysis but not when the dialyzed extract is heated. This hypothesis is in the process of being tested. Also, a thorough investigation of the relative stabilities of the wild-type and temperature-sensitive heterodimers in a wide temperature range and under various conditions of enzyme inhibition is under way. The main point of this preliminary report is to show that the same temperature-sensitive heterodimer can exhibit both positive and negative complementation.

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SUMMARY

Positive complementation is observed in the interaction of a temperature-sensitive subunit with a "wild-type" subunit in heterodimers when the hybrid enzyme is subjected to heat or dialysis. However, negative complementation resulting in high inactivation occurs when the heterodimer is heated after dialysis.

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