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BIOSYNTHESIS OF MAIZE ALCOHOL DEHYDROGENASE DIMERS: EVIDENCE FOR IMMATURE OLIGOMERIC FORMS

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SUMMARY

In vitro, alcohol:NAD$^+$ oxidoreductase (EC 1.1.1.1) monomers of maize associate into transient inactive oligomers. The immature aggregate is formed when the subunits reassociate at low temperature in the presence of zinc ions. A rapid transition occurs to the active oligomeric form when the temperature is elevated to 22–24 °C.

INTRODUCTION

The synthesis of the predominant alcohol:NAD$^+$ oxidoreductase in the maize kernels (EC 1.1.1.1) is under the control of the Adh_1 gene. The two common naturally occurring alleles are Adh_1^F and Adh_1^S. They specify electrophoretically distinguishable fast and slow migrating isozymes (Schwartz and Endo [1]). The enzyme behaves as a dimer and a hybrid FS isozyme is formed in the Adh_1^F/Adh_1^S heterozygotes in addition to the FF and SS isozymes which occur in the respective homozygotes. The F and S subunits dimerize randomly both in vivo and in vitro as determined from the ratio of intensities of the FF:FS:SS isozyme band intensities in Adh_1^F/Adh_1^S heterozygotes and in dissociation–reassociation experiments (Fischer and Schwartz [2]). The recovery of active enzyme following in vitro dissociation is zinc dependent suggesting that the maize alcohol dehydrogenase (ADH_1) is a zinc metalloprotein as has been shown for yeast (Vallee and Hoch [3]) and horse liver (Åkenson [4]) alcohol dehydrogenases.

Two pathways for the association of subunits into active oligomeric enzymes have been proposed (see Paulus and Alpers [5]). The subunits may first aggregate into inactive oligomers and then assume the thermodynamically stable active configurations. Alternatively, the subunit may first assume its thermodynamically most stable configuration and subsequently aggregate into active oligomers with no further conformational transitions, or assume the active configuration upon aggregation. According to the first scheme the oligomers should exist in two forms, a transient inactive form and a stable active form. For the alternative scheme all oligomers should be active. In this paper we present evidence for transient inactive oligomeric forms of

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alcohol dehydrogenase. The immature aggregate is formed when subunits reassociate at low temperature (4 °C) in the presence of zinc. At room temperature (22–24 °C) a rapid transition occurs to the active oligomeric form.

METHODS AND MATERIALS

Enzyme extracts were prepared by grinding dry kernels of the genotypes Adh_1^F/Adh_1^F and Adh_1^S/Adh_1^S in a Wiley Mill through a 20 mesh screen. 3 ml of dissociation buffer were added for each gram of meal. The slurry was intermittently stirred during incubation for 15 min at room temperature and then centrifuged for 15 min at 39 000 $\times$ g. The clear supernatants were collected and used in further experiments. Enzyme activity was determined by measuring the reduction of NAD^+ to NADH at 340 nm. A unit of enzyme activity represents a change in absorbance of 0.001 per min (Schwartz and Efron [6]). The dissociation and dialysis buffers were prepared according to Hart [7]. The dissociation buffer is composed of 0.1 M sodium phosphate buffer (pH 7.0), 1.0 M NaCl, 0.4 M sucrose and 0.1 M β-mercaptoethanol. The dialysis buffer is identical but NaCl is omitted. The dissociation of the alcohol dehydrogenase dimers was accomplished by freezing the enzyme extracts in dissociation buffer for 15–20 h at −20 °C. Following rapid thawing the frozen samples were either dialyzed at low temperature, room temperature, or incubated at room temperature following low temperature dialysis.

Starch gel electrophoresis and development of the alcohol dehydrogenase zymograms was according to Schwartz and Endo [1].

RESULTS AND DISCUSSION

FF and SS isozymes from Adh_1^F and Adh_1^S homozygous kernels were extracted in dissociation buffer and adjusted to equal activities (2800 units/ml). Extracts containing the FF enzyme were dissociated by freezing. After thawing, one aliquot was supplemented with $ZnCl_2$ to a final concentration of 7 μM and dialyzed against reassociation buffer containing $ZnCl_2$ at the same concentration. A second aliquot was treated in the same manner except that in the place of $ZnCl_2$, EDTA (sodium salt) was added to a final concentration of 10 mM to bind endogenous zinc. After 5 h of dialysis, enzymatic activity was measured. A portion of each dialyzed FF isozyme extract was mixed in a 1:1 ratio with non-dialyzed dissociated SS isozyme samples containing 7 μM $ZnCl_2$. The mixtures and the remaining dialyzed FF isozyme extracts were incubated at room temperature for 90 min. Enzyme activity was measured and the mixed extracts subjected to electrophoresis.

If, during the 4 °C dialysis of dissociated FF isozyme samples, the monomers readily reassociate into dimers only a small pool of F monomers should be available for reassociation with S monomers upon mixing. Zymograms of the subsequently reassociated mixtures should show a heterodimer FS isozyme band much weaker in intensity than the SS isozyme band. Alternatively, if the F monomers do not reassociate or reassociate only to a slight extent the pool of F monomers should remain large. Upon mixing and reassociation with S monomers, zymograms of the reassociated mixtures would be expected to show a strong heterodimer FS isozyme band with an intensity equal to or higher than the SS isozyme band.

In a typical experiment, after 5 h of dialysis of the FF isozyme sample at 4 °C in the presence of $ZnCl_2$, the measured activity was 800 units/ml. After subsequent room temperature incubation for 90 min, the activity increased to 2450 units/ml. Thus in the cold only 33% of the recoverable enzyme was in the active dimer form. If the remaining 67% was in the form of monomers, mixing with dissociated S subunits should produce a prominent FS isozyme heterodimer band. However, the zymogram (Figs 1a and 2a) patterns do not conform with this expectation. The heterodimer band is very weak in comparison with the FF and SS isozyme homodimer bands. The

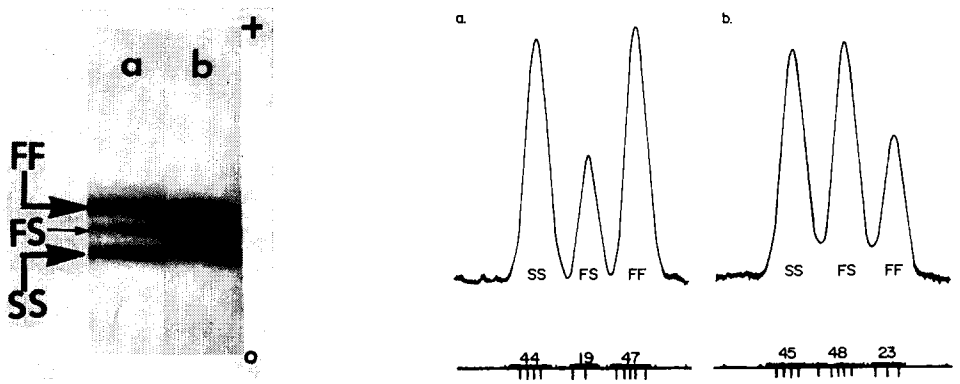

Fig. 1. Zymograms of reassociated mixtures of FF and SS dissociated samples. The FF isozyme extract was dissociated, dialyzed at 4 °C for 5 h then mixed with dissociated S monomers. The mixtures were incubated for 90 min at room temperature. O = origin. (a) Pattern of reassociated mixture of S isozyme with $ZnCl_2$ supplemented F isozyme. (b) Pattern of reassociated mixture of S isozyme with EDTA-treated F isozyme.

Fig. 2. Densitometer tracings of zymograms shown in Fig. 1. (a) zinc supplemented; (b) EDTA treated.

relative intensities of the FF and SS isozyme bands measured in a densitometer for the zinc-treated extract is 1 FS:2.3 SS. This ratio is expected when the number of F monomers available for reassociation is only about 20% of the S monomers. The high intensity of both the FF and SS isozyme homodimer bands indicates that most of the F monomers were already reassociated into dimers prior to mixing with the dissociated S isozyme. Although only 33% of total recoverable activity is measured after 4 °C dialysis, about 80% of the F subunits were already in the form of dimers. If dimerization of maize alcohol dehydrogenase monomers is accompanied by an immediate recovery of catalytic activity, 80% of recoverable activity would be expected after 4 °C dialysis. This leads to the conclusion that a substantial fraction of the FF enzyme (80% − 33% = 47%) was in an inactive form. The transition of these immature forms into stable active dimers occurs upon incubation at room temperature. Indeed a very rapid increase in enzyme activity is observed during the first 10 min after transfer to room temperature (Fig. 3).

A strikingly different situation was observed in the EDTA-treated, cold dialyzed sample. Enzymatic activity after 5 h of dialysis was only 150 units/ml. In contrast to the zinc-supplemented sample, considerably more of the recovered enzyme was in the form of monomers at the time of mixing with the dissociated S extract,

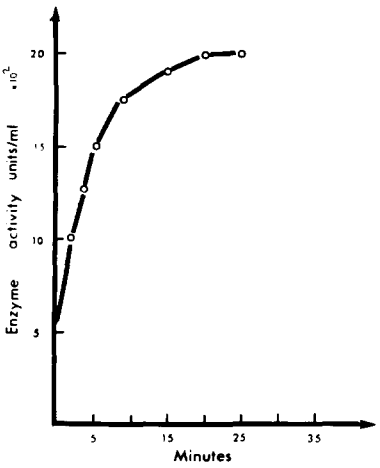

Fig. 3. Reactivation of dissociated cold dialyzed FF enzyme upon transfer to room temperature.

since the concentration of FF isozyme homodimers in the room temperature incubated mixture is much less than that of the FS isozyme heterodimer (Figs 1b and 2b). The difference in FS:FF isozyme ratio in the two mixture samples is clearly seen when the densitometer tracings of the zymograms are compared.

This work provides strong evidence for the occurrence of immature forms of maize alcohol dehydrogenase dimers. These intermediates are probably inactive but the results are also consistent with the possibility that they may have some low level of activity. Since preliminary experiments with purified FF and SS isozymes give similar results, the analysis and characterization of these immature forms may be possible.

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