

Thrombin-Induced Release of Calcium from Blood Platelets

Abstract. Washed blood platelets, suspended in buffered saline, release most of their calcium to the surrounding medium when they are incubated with thrombin at 37°C. The release is inhibited when energy metabolism is blocked.

Blood platelets contain 3.0 to 4.5 mg of calcium per gram (dry weight) (1). The function of this great concentration of calcium in the platelets is still unknown, although studies of other cell types reveal a necessity for Ca^{2+} in different release reactions (2). At the same time, an increased extracellular concentration of ethylenediaminetetraacetic acid (far above that necessary to form a complex with the platelets' total calcium content) does not interfere with the release of adenine nucleotides induced by thrombin, latex particles, or sodium fluoride (3).

To understand the role of the platelets' calcium store, it is of interest to ascertain whether calcium is released under the same conditions as the other stored compounds in platelets (for example, adenine nucleotides and serotonin).

The experiments were performed with platelets from normal human donors or from a patient with hemochromatosis, a disease which should not interfere with the normal function of platelets. Conditions for isolation and washing (twice) of platelets, as well as for the performance of the experiments, have been described earlier for studies of adenine nucleotide release (3).

Table 1. Release of calcium from washed platelets from normal human donors. After a 20-minute incubation period without thrombin, the washed platelets were incubated at 37°C for 5 minutes with or without bovine thrombin in 4 ml of a medium consisting of 135 mM NaCl, 3 mM ethylenediaminetetraacetic acid, and 25 mM tris(hydroxymethyl)aminomethane buffered to pH 7.4 with HCl. The suspension was centrifuged for 20 minutes (1000g) after being cooled, the supernatant was decanted, the pellet was dissolved in 0.2 ml of 5 percent deoxycholate and 17.5 percent KOH and diluted with water to 2 ml, and the calcium content of supernatant and pellet were measured in a Unicam atomic absorption spectrophotometer (6).

Protein (mg)	Thrombin (5 NIH units)	Inhibitors*	Calcium	
			Supernatant (μg)	Pellet (μg)
4.5	0	0	1.2	9.6
4.5	+	0	6.8	2.4
9.0	0	0	1.2	16.8
9.0	+	0	11.6	3.0
9.0	+	+	4.4	13.2

* Inhibitors were 250 ng of antimycin and 25 μmole of 2-deoxy-D-glucose.

In experiments with five different platelet preparations the calcium concentration in the supernatant after incubation varied from 7 to 29 percent of the total in the absence, and from 77 to 88 percent in the presence, of thrombin (Table 1). Most of the calcium in platelets is released by the action of thrombin, and the release is inhibited by the combined inhibitors of energy metabolism. The function of this release may be the same as the function of the release of adenine nucleotides, namely, to make the platelets "sticky" and thus perform their role in hemostasis (4). Another function may be to act as counterion to the adenine nucleotides in the storage and transport of these.

tides in the storage and transport of these.

There is evidence (1) that most of the platelets' calcium is not exchangeable with extracellular Ca^{2+} . It is therefore very likely that the calcium is stored in the granules in the same manner as that proposed for adenine nucleotides (5).

E. H. MÜRER

Institute for Thrombosis Research, Rikshospitalet, Oslo 1, Norway

References and Notes

1. A. J. Marcus and M. B. Zucker, *The Physiology of Blood Platelets* (Grune & Stratton, New York, 1965).
2. H. Stormöken, *Scand. J. Hematol.*, in press.
3. E. H. Mürer, *Biochim. Biophys. Acta* **162**, 320 (1968).
4. A. Gaarder, J. Jonsen, S. Laland, A. Hellem, P. A. Owren, *Nature* **192**, 531 (1961).
5. H. Holmsen, H. J. Day, E. Storm, *Biochim. Biophys. Acta* **186**, 286 (1969).
6. Spectrophotometer, courtesy of Institute of Clinical Biochemistry, University Hospital, Oslo.

23 June 1969; revised 29 July 1969

Alcohol Dehydrogenase in Maize: Genetic Basis for Isozymes

Abstract. There are two distinct electrophoretic forms of alcohol dehydrogenase (alcohol:NAD oxidoreductase, E.C. 1.1.1.1) in each of several inbred strains of maize examined. Electrophoretic variants of each form are under genetic control. Recombinants recovered from crosses involving the variant isozymes suggest that the two isozymes are under the control of two distinct but closely linked loci.

Inbred strains of maize contain two major alcohol dehydrogenases (ADH) which on starch gel electrophoresis, at pH 8.0, migrate anodally, one faster and the other slower. I have termed these ADH-1 and ADH-2, respectively (1). In each zone, two variants are known, a relatively fast and a relatively slow one. Usually, a maize strain contains either the fast variants of ADH-1 and of ADH-2 or else the slow variants of either enzyme. This phenotypic situation has been explained (1) by assuming two loci, *Adh-1* and *Adh-2*, each existing as two alleles, with *Adh-1^F* specifying the fast variant and *Adh-1^S* the slow variant of the ADH-1 zone, and *Adh-2^F* and *Adh-2^S* specifying the fast and slow variants, respectively, of the ADH-2 zone. To account for the usual correlation in the relative electrophoretic mobilities of the ADH-1 and ADH-2 variants, a close linkage between *Adh-1* and *Adh-2* has been assumed, *Adh-1^F* normally being linked with *Adh-2^F*, and *Adh-1^S* with *Adh-2^S*. The allelic products of the *Adh-2* gene interact to generate a hybrid molecule in the heterozygote, in addition to the parental type molecules; how-

ever, there is no hybrid molecule generated in the F_1 relative to *Adh-1*. These findings suggest that ADH-2 exists functionally as a dimer, and the hybrid enzyme formed in the ADH-2 zone of heterozygotes may be the result of random association of two different ADH-2 monomeric subunits. The

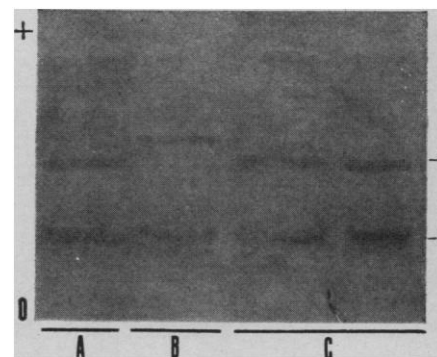


Fig. 1. Zymogram of F_2 kernels ($FS \times FS$) assayed for ADH activity 16 days after pollination. A and C are phenotypes normally expected from an F_2 cross (*Adh-1^S/Adh-1^S*; *Adh-2^S/Adh-2^S*). However, B shows a phenotype which could only arise as a result of crossing-over, the phenotype being (*ADH-1^F/ADH-2^S*). Numbers on the right indicate the ADH zones.

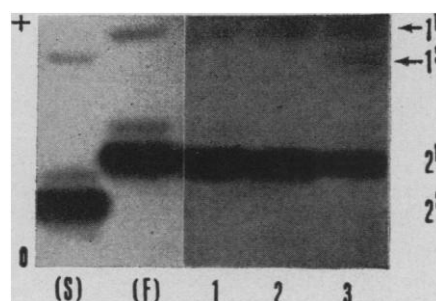
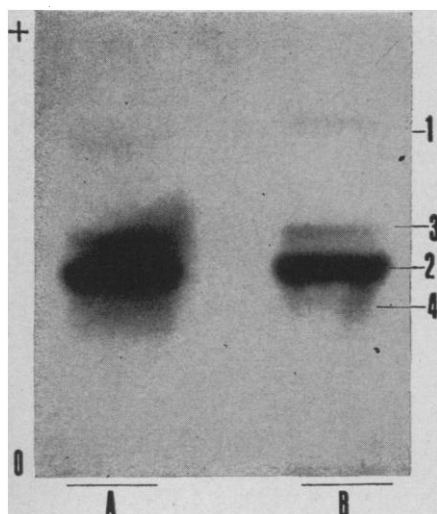


Fig. 2 (left). ADH zymogram of scutella taken from mature F_2 kernels after soaking for 36 hours. (A) Rare recombinant-type kernel having the phenotype ($ADH-1^S/ADH-2^F$). (B) A normally expected ADH phenotype from an F_2 cross ($ADH-1^F/ADH-2^F$). Numbers on the right indicate the ADH zones. Zones 3 and 4 are temporal, being present only in early stages

of germination or in scutella of fully matured kernels; no electrophoretic variants of zones 3 and 4 have been detected. Fig. 3 (right). Zymograms of scutella taken from F_2 kernels 35 days after pollination. The two kernels to the left have the phenotype ($ADH-1^F/ADH-2^F$). Note the recombinant kernel to the right, having the phenotype ($ADH-1^{FS}/ADH-2^F$). F and S , given for comparison, show the inbreds having the phenotypes $ADH-1^F$; $ADH-2^F$ (F) and $ADH-1^S$; $ADH-2^S$ (S).

absence of a hybrid enzyme in the $ADH-1$ zone of F_1 kernels can most simply be explained to mean that $ADH-1$ exists functionally as a monomer, or as a dimer resulting from interaction of identical subunits. There is no molecular interaction between the products of $Adh-1$ and $Adh-2$ genes in vivo which results in detectable hybrid molecules.

Evidence for the suggested two-loci hypothesis comes mainly from the recovery of three recombinants in the F_2 progeny which resulted from self-pollinating F_1 plants ($Adh-1^F Adh-2^F/Adh-1^S Adh-2^S$). Over a period of several years we examined 20,124 F_2 kernels for ADH activity at various stages of development as part of our efforts

to study the epigenetics and chemistry of these isozymes. It was among this large number of F_2 progeny scored that the three aberrant phenotypes were found. One of the three recombinants was found among F_2 kernels examined 16 days after pollination (Fig. 1).

The other two recombinants were found on examining scutellar extracts of F_2 kernels (Figs. 2 and 3). All three aberrant ADH zymogram patterns recovered ($ADH-1^F/ADH-2^S$; $ADH-1^S/ADH-2^F$; and $ADH-1^{FS}/ADH-2^F$) fit the theoretical phenotypes expected from an F_2 cross, assuming crossing-over occurs (Fig. 4). The frequency with which they are recovered is indeed low but not without

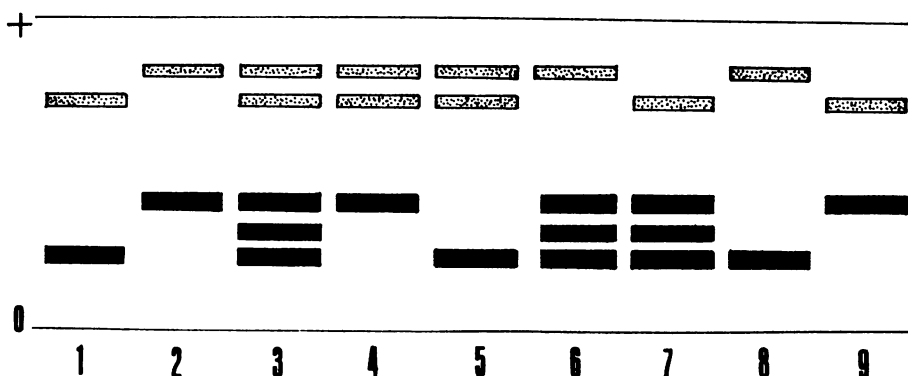


Fig. 4. Schematic zymogram showing the theoretical ADH phenotypes expected from an F_2 cross ($FS \times FS$), assuming crossing-over occurs. (1) SS/SS ; (2) FF/FF ; (3) FF/SS ; (4) FF/SF ; (5) FS/SS ; (6) FF/FS ; (7) SF/SS ; (8) FS/FS ; (9) SF/SF . The recombinants actually recovered (Figs. 1, 2, and 3) correspond to numbers 8, 9, and 4, respectively.

precedent (2), which suggests extremely close linkage. One of the recombinants has been briefly mentioned before (3).

Schwartz has proposed (4) an alternative explanation that both $ADH-2$ (his Set I) and $ADH-1$ (his Set II) are products of the same genetic locus (his Adh_1). In addition, to explain the differences in electrophoretic mobility between $ADH-1$ and $ADH-2$, he proposes the existence of a second gene (his Adh_2) which presumably codes for inactive ADH_2 polypeptides. He postulates that the inactive polypeptides of his Adh_2 locus interact with a subunit of the Set I gene product, resulting in the distinct electrophoretic position of the Set II isozymes. Schwartz reports (5) that his hypothesis was essential to explain the usual correlation between the fast and the slow forms of the two enzymes, a correlation he considers complete.

Schwartz reports (5) that he induced mutations affecting a concomitant and correlated change in the electrophoretic position of the zone 1 and zone 2 ADH isozymes by treating homozygous kernels with ethyl methanesulfonate. He reports the recovery of four such kernels but does not state the frequency with which they occurred. While adding support for his hypothesis, he does not explain how it might account for results such as ours, that is, the occurrence of kernels in which the usual correlation between the fast and the slow variants of either ADH enzyme has been disrupted. His statement that "the Adh_2 mutations which should have altered the migration rate of only the Set II isozyme were not found" is not very meaningful without precise numerical data. In fact, as long as he cannot provide evidence for mutations of his Adh_2 locus, the existence of this locus is not supported. The results reported here lend support to the two gene hypothesis of ADH control in maize.

JOHN G. SCANDALIOS
MSU/AEC Plant Research Laboratory,
Michigan State University,
East Lansing 48823

References and Notes

1. J. G. Scandalios, *Biochem. Genet.* 1, 1 (1967).
2. E. Bahn, *Hereditas* 58, 1 (1967).
3. J. G. Scandalios, *Biochem. Genet.* 3, 37 (1969).
4. D. Schwartz, *Proc. Nat. Acad. Sci. U.S.A.* 56, 1431 (1966).
5. —, *Science* 164, 585 (1969).
6. I thank R. G. Sculley for technical assistance. Supported by AEC contract No. AT(11-1)-38. 3 July 1969; revised 18 August 1969