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 m*M* NaCl, 3 m*M* ethylenediaminetetraacetic acid, and 25 m*M* tris(hydroxymethyl) aminomethane buffered to *p*H 7.4 with HCl. The suspension was centrifuged for 20 minutes (1000*g*) 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.

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*).

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References and Notes

1. A. J. Marcus and M. B. Zucker, *The Physiology of Blood Platelets* (Grune & Stratton, New York, 1965).
2. H. Stormorken, *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 *p*H 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; however, 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

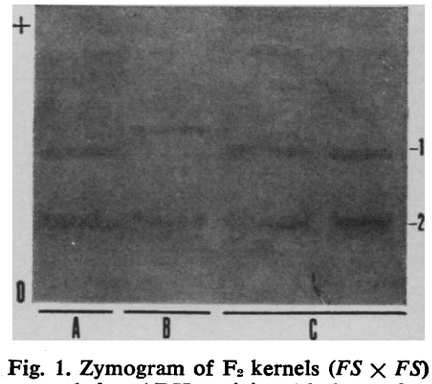

Fig. 1. Zymogram of F_2 kernels (*FS* × *FS*) assayed for ADH activity 16 days after pollination. *A* and *C* are phenotypes normally expected from an F_2 cross (*Adh-1*^{S}/*Adh-1*^{S}; *Adh-2*^{S}/*Adh-2*^{S}). However, *B* shows a phenotype which could only arise as a result of crossing-over, the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