Influence of Apyrase on Stability of Suspensions of Washed Rabbit Platelets (35419)

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The preparation of suspensions of washed platelets from rabbits has been described elsewhere (1). These suspensions aggregate and disaggregate upon the addition of the low concentrations of ADP that are effective in plasma and, when stored at room temperature (20–24°), maintain their ability to respond to ADP for several hours. This report describes the stabilizing effect of potato apyrase on these suspensions which allows them to be stored at 37°.

Materials and Methods. Potato apyrase (Grade 1) (ATP diphosphohydrolase 3.6.1.5.) was obtained from Sigma Chemical Co., St. Louis, Mo. All of these studies were done with lot No. 89B-5100 (approx activities/mg: 5′-ATPase, 1.5 units; 5′-ADPase, 0.35 units; 5′-AMPase, 0.03 units). No other lots were used in these experiments, but an occasional batch (lot No. 99B-5030) has been received that varied considerably from these activities. The type of potato used has been found to affect the relative activity of the enzyme on ATP and ADP (2).

Suspensions of twice-washed rabbit platelets in Tyrode's solution containing 0.35% bovine albumin were prepared as described previously (1). Apyrase, at concentrations ranging from 0 to 1 mg/ml was added to the final suspending fluid before the platelets were resuspended in it. Suspensions were stored at 37°. Aggregation was studied by a turbidimetric method (3).

The rate of conversion of ^{14}C-ADP (adenosine-8-^{14}C 5′-diphosphate, trisodium salt, New England Nuclear Corp., Boston, Mass.) to other compounds was determined following paper chromatography and liquid scintillation counting of perchloric acid-treated samples of supernatants from the platelet suspension (4).

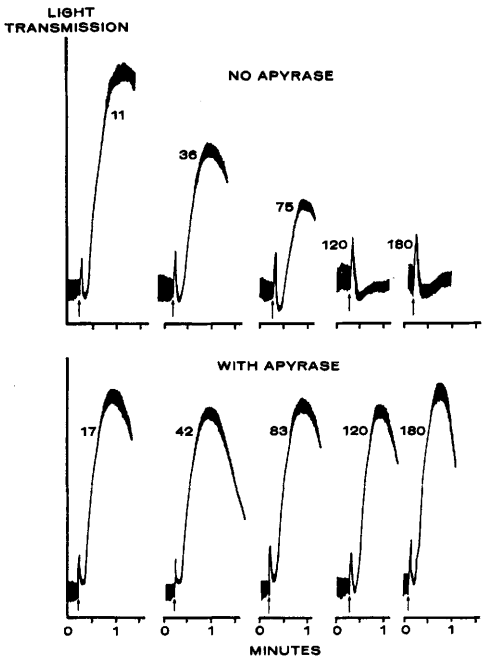

FIG. 1. Response to ADP of washed rabbit platelets stored at 37° without apyrase (upper portion); or with apyrase (lower portion): apyrase, 100 μg/ml; ADP, 4.5 × 10^{-7} *M*; platelet count, 750, 000/mm^3. Beside each aggregation curve is shown the number of minutes of storage before ADP addition (indicated by arrows) (typical of 5 expts. that gave similar results).

Results. Suspensions of washed rabbit platelets stored at 37° gradually lost their sensitivity to ADP-induced aggregation (Fig. 1). When apyrase was included in the suspending medium, their sensitivity to ADP was maintained for at least 3 hr at 37° (Fig. 1).

TABLE I. Effects of Different Concentrations of Apyrase on Maintaining the Sensitivity to ADP of Washed Rabbit Platelets[a] Stored at 37°.

Conc of apyrase in suspending medium (μg/ml)	Maximum increase in light transmission[b] after ADP[c] addition (min)[d]				
	Initial[e]	30	60	120	180
0	22.5	14.4	4.3	0	0
50	22.8	17.8	17.7	14.2	5.9
100	24.5	17.4	19.3	14.5	9.9
150	20.6	17.6	16.8	13.4	9.5
200	19.6	17.9	16.0	11.9	9.0
400	13.6	14.3	14.5	13.5	10.3

[a] Platelet count, 500,000/mm³.

[b] The values shown are the maximum heights of the aggregation curves (cm).

[c] Final concentration: 9.1×10^{-6} *M*.

[d] Times indicate number of minutes of storage at 37° before response to ADP was tested.

[e] This refers to the time at which the apyrase was added to the suspension at 37°. Successive samples were started at 2-min intervals (typical of 4 expts. that gave similar results).

Table I shows the effects of different concentrations of apyrase on maintaining the sensitivity to ADP of washed rabbit platelets stored at 37°. An apyrase concentration of 100 μg/ml was most satisfactory. The lower concentration did not maintain platelet sensitivity and the highest concentration probably degraded the added ADP so quickly (Table II) that the extent of aggregation was reduced.

Table II shows the effect of various concentrations of apyrase on the rate of breakdown of ^{14}C-ADP. Apyrase, at a concentration of 1000 μg/ml, had degraded all the ^{14}C-ADP within 1 min. Although ^{14}C-ADP was initially converted to ^{14}C-AMP, further degradation also occurred in 5 min, with the highest concentration of apyrase.

Incubation of platelets at 37° in the presence of ADP in concentrations between 10^{-7} and 10^{-8} *M* accelerated the loss of platelet sensitivity to ADP-induced aggregation, but if apyrase were present also, this decrease in sensitivity did not occur (Table II). Platelets (stored without apyrase at 37°) that had become insensitive to ADP-induced aggregation could be partially restored by incubation with pyrase (Table

TABLE II. Rate of Breakdown of ^{14}C-ADP[a] in Tyrode's Albumin Solution by Various Concentrations of Apyrase.

Conc of apyrase (μg/ml)	Labeled compounds	Percentage of ^{14}C in chromatography lane in ^{14}C labeled compounds (min): 0	1	3	5
0	ADP	100	100	100	100
	AMP	0	0	0	0
10	ADP	100	97	92	86
	AMP	0	3	8	14
100	ADP	100	61	23	9
	AMP	0	39	77	91
1000	ADP	100	0	0	0
	AMP	0	79	58	35

[a] ^{14}C-ADP, 1.16×10^{-5} *M*.

TABLE III. Effect of Apyrase on the Refractory State of Rabbit Platelets[a] Caused by the Presence of ADP in the Suspending Fluid.

Conc of ADP incubated with platelets (M)	Time of incubation (min)	Maximum increase in light transmission[b]; incubation	
		Without apyrase	With apyrase (100 μg/ml)
0	10	14.3	15.6
10^{-7}	10	10.2	13.4
5×10^{-7}	10	2.2	11.4
10^{-6}	10	0.3	9.6
0	30	7.8	14.2
10^{-7}	30	3.7	10.3
5×10^{-7}	30	0.1	10.8
10^{-6}	30	0	10.8
0	60	0.7	13.1
10^{-7}	60	0.2	10.6
5×10^{-7}	60	0	11.7
10^{-6}	60	0	11.2
0	90[c]	2.1	
10^{-7}	90[c]	3.6	
5×10^{-7}	90[c]	2.3	
10^{-6}	90[c]	2.0	

[a] Platelet count 500,000/mm^3.

[b] Following addition of ADP, 9.1×10^{-6} M, with stirring.

[c] Last 30 min with apyrase, 100 μg/ml (typical of 3 expts. that gave similar results).

III). This was observed both with suspensions to which no ADP had been added, and with those that had been incubated with ADP.

Discussion. Washed rabbit platelets stored at 37° gradually lose their ability to aggregate upon the addition of ADP. This loss of sensitivity occurs more rapidly if ADP is added to the platelet suspensions before storage. Apyrase in the platelet suspending fluid degrades added ADP and would degrade any ATP and ADP that might be lost from the platelets; thus the addition of apyrase prevents exposure of the platelets to these nucleotides. The lack of ADP in the suspending fluid is probably responsible for the maintenance of their responsiveness because it is well established that platelets stored in the presence of ADP become refractory and will not aggregate upon the addition of more ADP with stirring (4–7). It seems likely that the loss of sensitivity of platelets stored without apyrase at 37° is caused, at least in part, by the gradual accumulation of adenine nucleotides in their suspending medium.

Summary. The addition of apyrase to suspensions of washed rabbit platelets stored at 37° maintains their sensitivity to ADP-induced platelet aggregation. This appears to be due to the degradation of the ADP which accumulates; if not degraded, this ADP can make the platelets refractory to added ADP.

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