CORRESPONDENCE

Human Erythrocyte Membrane Enzymes

To the Editor:

An increasing knowledge has accumulated regarding the metabolic events within red blood cells, the frontier of investigation has gradually shifted to the red cell membrane. Schrier's review[1] in this journal concerning the association of certain enzymes with the red cell membrane was informative and timely.

We were interested to learn that many acid glycohydrolases, such as α-galactosidase, β-hexosaminidase, and β-glucosidase, have been reported to be associated with the red cell membrane. The 1971 article by Bosmann[2] that made this claim had escaped our attention. The presence of these enzymes in the membrane would, indeed, be of considerable interest. As Schrier pointed out, it might suggest, for example, the participation of lysosomal remnants in the formation of the membrane. Moreover, since deficiencies of these enzymes are responsible for hereditary storage diseases such as Fabry, Tay-Sachs, and Gaucher diseases, red cells might provide a ready means for the diagnosis of these disorders. However, in our studies of the biochemical genetics of glycolipid storage diseases we have found it necessary to use leukocytes or fibroblasts, since we have consistently found red cells to be lacking in the appropriate glycohydrolase activity.

Recently, we discovered that the activity of another enzyme reported to be present in red cell membranes, γ-glutamyl transpeptidase, resided, in point of fact, in leukocytes.[3] It seemed that the results reported by Bosmann, too, might have been due to leukocyte contamination of the red cell membrane preparations.

Accordingly, we systematically examined the activity of acid hydrolases in red cell membranes prepared by the method[4] employed by Bosmann. Studies were carried out on two types of red cell preparations. The first preparation was made from blood washed six times with removal of the buffy coat; the second consisted of red blood cells obtained from blood freed almost entirely of leukocytes and platelets by defibrinating and passing the blood through an α-cellulose–microcrystalline cellulose column.[5] Acid glycohydrolase assays were performed using 4-methylumbelliferone substrates.[6]

The results of these investigations are shown in Table 1. In several cases *p*-nitrophenol derivatives were used as substrates, with similar results. Stroma preparations made from leukocyte-free blood manifested no significant activity of any of the acid glycohydrolases measured, except for β-glucuronidase. With that exception, the small numbers of residual leukocytes present even in the filtered red cells could easily account for the minimal activities found.

Table 1. Acid Glycohydrolases in Red Cell Membrane Preparations

Enzyme	RBC Membranes Prepared as Described by Danon et al.[4]*	RBC Membranes Prepared After Leukocyte and Platelet Removal†
β-Glucosaminidase	3.14	0.05
β-Glucosidase	ND	ND
β-Galactosidase	0.31	0.01
β-Glucuronidase	0.98	0.25
α-Glucosidase	0.34	ND
α-Mannosidase	1.15	0.01
α-Galactosidase	0.33	0.01

Activity is expressed as mU/mg protein. ND, not detected.

*Red cells were washed six times with 0.9% saline. Membranes were prepared by adding 1 volume of red cells to 40 volumes of 60 mM NaCl in phosphate buffer, and by successively washing with phosphate-buffered NaCl in 15 mM decrements.

†Blood was defibrinated and passed over microcrystalline cellulose-α-cellulose[5] before washing with 0.9% saline and proceeding as in the footnote above.

It is apparent from these and earlier investigations that leukocyte contamination is a particularly important consideration in the study of red cell membranes. Leukocyte debris sedimented with red cell stroma in the process of preparing membranes may result in erroneous interpretation of the origin of enzymatic activity in red cell membrane preparations.

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REFERENCES

1. Schrier SL: Human erythrocyte membrane enzymes: Current status and clinical correlates. Blood 50:227, 1977

2. Bosmann HB: Glycosidase activities of human erythrocyte plasma membranes. J Membrane Biol 4:113, 1971

3. Srivastava SK, Awasthi YC, Miller SP, Yoshida A, Beutler E: Studies on γ-glutamyl transpeptidase in human and rabbit erythrocytes. Blood 47:645, 1976

4. Danon D, Nevo A, Marikovsky Y: Preparation of erythrocyte ghosts by gradual haemolysis in hypotonic aqueous solution. Bull Res Council Isr 6:36, 1956

5. Beutler E, West C, Blume KG: The removal of leukocytes and platelets from whole blood. J Lab Clin Med 88:328, 1976

6. Beutler E, Kuhl W, Matsumoto F, Pangalis G: Acid hydrolases in leukocytes and platelets of normal subjects and in patients with Gaucher's and Fabry's disease. J Exp Med 143: 975, 1976

Human Erythrocyte Membrane Enzymes: Reply

To the Editor:

Dr. Beutler and Dr. Kuhl in the preceding letter touch upon an important point I did not emphasize sufficiently in my review. Where an enzyme activity thought to be in a membrane fraction is present in only trace amounts, it is of course possible that contaminating leukocytes or platelets might be the source of the enzyme, rather than the erythrocyte membrane.

Partly because of the considerations raised by Beutler and Kuhl, I did not include in the review a discussion of membrane-associated enzymes in reticulocytes, since the separatory techniques for isolating reticulocytes also increase the chance of concentrating leukocytes and leukocyte fragments. We have used the filtration technique described by Dr. Beutler and have found it to be useful in exploring the possibility of leukocyte contamination.

Before one decides that a trace membrane enzyme belongs to the erythrocyte membrane, one probably should study membranes prepared from erythrocytes filtered through microcrystalline cellulose-α-cellulose. One purpose of a review is to stimulate critical research. I am delighted that Dr. Beutler and Dr. Kuhl have responded so promptly.

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