Journal of Bioenergetics and Biomembranes, Vol. 14, No. 4, 1982

Iron and Copper in Plasma Membranes

W. C. MacKellar[1] and F. L. Crane[1]

Received January 11, 1982

Abstract

Nonheme iron has been found in pig erythrocyte and mouse liver plasma membranes. The amount found, 8.2 nmol/mg protein in erythrocyte membranes and 7.4 nmol/mg protein in liver plasma membrane, is slightly lower than values reported for endoplasmic reticulum and Golgi apparatus. Less than one-third of the erythrocyte membrane iron can be released by acid treatment, which indicates that most of it is not in the typical iron–sulfur structure. Copper has been found in pig erythrocyte plasma membrane at a concentration of 0.45 nmol/mg protein. These metals may be associated with the redox enzymes of plasma membranes.

Key Words: Plasma membrane; iron; copper; chelator.

Introduction

Plasma membranes from several different types of cells have been found to contain redox enzymes. Among the enzyme activities reported are NADH oxidase, glutathione oxidase, and xanthine oxidase (Crane *et al.*, 1979; Jarasch *et al.*, 1977; Ormstad *et al.*, 1979). Fatty acid desaturase activity has also been reported in plasma membranes (Giacobino and Chmelar, 1977). The presence of redox enzymes such as these indicates that plasma membranes can be expected to contain significant quantities of nonheme iron or other metals. We have examined mouse liver and porcine erythrocyte plasma membranes and find that they contain nonheme iron and copper.

Materials and Methods

Membranes were prepared by the following method: Golgi apparatus and endoplasmic reticulum by the method of Morré et al., 1972, and Morré, 1973; mouse liver plasma membrane by the procedure of Goldenberg *et al.* (1979).

[1]Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907.

0145-479X/82/0800-0241$03.00/0

Porcine erythrocyte plasma membrane was prepared from fresh blood treated with 15 mM ethylenediaminetetraacetate (EDTA) to prevent coagulation. Membranes were prepared by a modified procedure of Dodge *et al.*, 1963. Erythrocytes were isolated by centrifugation for 10 min at 3000 × *g*. The plasma and buffy coat were removed by aspiration. The cells were washed a total of three times in 109 mM sodium phosphate buffer, pH 7.5.

Hemolysis was performed by the following procedure: 1 ml of packed cells was pipetted into 50 ml of 3.6 mM sodium phosphate buffer, pH 7.5. The lysate was centrifuged at 20,000 × *g* for 10 min and the supernatant was aspirated along with the hard button under the loose membrane pellet. The membranes were washed three times in the same buffer, or until they were visibly free of hemoglobin. The membranes were prepared for metal determinations by first suspending them in 1.5 m KCl, 10 mM Tris-HCl buffer, pH 7.4, and incubating for 15 min at o°C. This suspension was centrifuged at 20,000 x *g* for 10 min and the supernatant was removed. The pellet was suspended in 1 mM EDTA, pH 7.5, and incubated for 15 min at o°C. This solution was centrifuged as above and the EDTA treatment was repeated on the pellet, substituting 0.1 M EDTA for 1 mM EDTA. Again the solution was centrifuged and the pellet was washed with distilled water (Chelex treated). The final pellet was resuspended in distilled water (Chelex treated).

The iron and copper determinations were performed according to the method of Beinert (1978) with all membrane ashing by the peroxide procedure.

Dehydrogenase activities were measured with an Aminco DW-2a spectrophotometer in the dual-beam mode. The reaction mixture for ferricyanide reduction contained 50 mM sodium phosphate buffer, pH 7.0, 0.12 mM NADH or NADPH, 200 μM potassium ferricyanide, and inhibitors as indicated, in a total volume of 3.0 ml. The reaction was followed at a wavelength of 420 nm against a 500 nm reference. The conditions for cytochrome *c* reduction were the same as for ferricyanide reduction except 100 μg/ml of cytochrome *c* was substituted for ferricyanide. This reaction was followed at 550 nm vs. 541 nm reference. Extinction coefficients used for calculation of ferricyanide and cytochrome *c* reduction were 1 and 19 $mM^{-1}cm^{-1}$, respectively. NADH and ascorbate oxidase- and vanadate-stimulated NADH oxidase were measured according to Crane *et al.* (1980). Cytochrome spectra were measured as described by Bruder *et al.* (1980).

NADH, NADPH, succinate, beef heart cytochrome *c*, bathophenanthroline sulfonate, and thioglycolic acid were obtained from Sigma Chemical Co. Vanadium pentoxide was from K and K Chemical Co., and Chelex 100 resin was from Bio-Rad (Richmond, California). All other chemicals were analytical grade reagents.

Table I. Redox Activities of Plasma Membrane with Various Electron Acceptors and Electron Donors[a]

Activity	Plasma membrane	
	Porcine erythrocyte	Mouse liver
NADH → Ferricyanide	241	500
NADPH → Ferricyanide	0	36
NADH → Cytochrome *c*	3.3	40
NADH → O_2	0	50
NADH → Vanadate (O_2)	163	196
Ascorbate → O_2	0	140
Succinate → Cytochrome *c*	0	3

[a]Activity is expressed in nanomoles acceptor reduced per minute per milligram protein. The ascorbate oxidase and NADH oxidase activity are not inhibited by 10^{-3} M KCN or stimulated by cytochrome *c*. The NADH cytochrome *c* reductase activity is not inhibited by 1 μM rotenone.

Results

Dehydrogenase and oxidase activity in plasma membrane preparations from porcine erythrocytes and mouse liver are shown in Table I. Both membrane have high levels of NADH ferricyanide reductase activity and vanadate-stimulated NADH oxidase. The erythrocyte membrane has much lower NADH cytochrome *c* reductase activity than liver membrane and completely lacks NADH oxidase activity. NADPH dehydrogenase is completely lacking in the erythrocyte membranes, whereas a small amount is detected in the liver plasma membranes. This may be endogenous to liver plasma membrane and clearly shows minimum contamination by endoplasmic reticulum. Low succinate cytochrome *c* reductase shows low contamination of the preparations with mitochondria (Goldenberg *et al.*, 1979).

Both the mouse liver and porcine erythrocyte plasma membranes contain

Table II. Iron Content of Plasma Membranes, Endoplasmic Reticulum, and Golgi Apparatus Membranes[a]

	Total iron	Cytochromes
	nmol/mg protein	
Pig erythrocyte plasma membrane	8.2 ± 2.2 (8)	0.06
Pig erythrocyte plasma membrane after 1 N HCl extraction	4.8 ± 1.3 (3)	—
Mouse liver plasma membrane	7.4 ± 2.8 (5)	0.45
Mouse liver endoplasmic reticulum	10.8 ± 1.3 (4)	1.44
Mouse liver Golgi membranes	11.6 ± 3.6 (4)	0.6

[a]Values given with standard error of mean for the number of membrane preparations in parentheses.

significant amount of nonheme iron (Table II). The nonheme iron content in erythrocyte membrane is especially high in relation to the cytochrome iron.

The cytochrome spectrum of the porcine erythrocyte plasma membrane is similar to that found in chicken, rat, and human erythrocytes with peaks at 428 and 556 nm (Fig. 1). At −196°C the α peak separates into three components at 552, 557.5, and 560 which have been interpreted by Bruder *et al.* (1980) to represent cytochrome b_5 and P420. On the basis of their calculation procedure, we find 0.024 nmol cytochrome b_5/mg protein and 0.036 nmol P420/mg protein in the pig erythrocyte membrane.

The copper content of the porcine erythrocyte membrane is much lower than the iron content (Table III). The value which we find is somewhat lower than values reported for rat liver plasma membranes and microsomes, but is close to values found in rabbit liver microsomes.

Incubation of erythrocyte membrane with the iron chelator, bathophenanthroline sulfonate, in the assay buffer for 2 min prior to starting the reaction causes an inhibition of both the NADH ferricyanide and the NADH cytochrome *c* reductase activity (Fig. 2).

Discussion

It is apparent that the total iron content of plasma membranes is 10 to 100 times the heme iron content. Similar proportions of total iron and

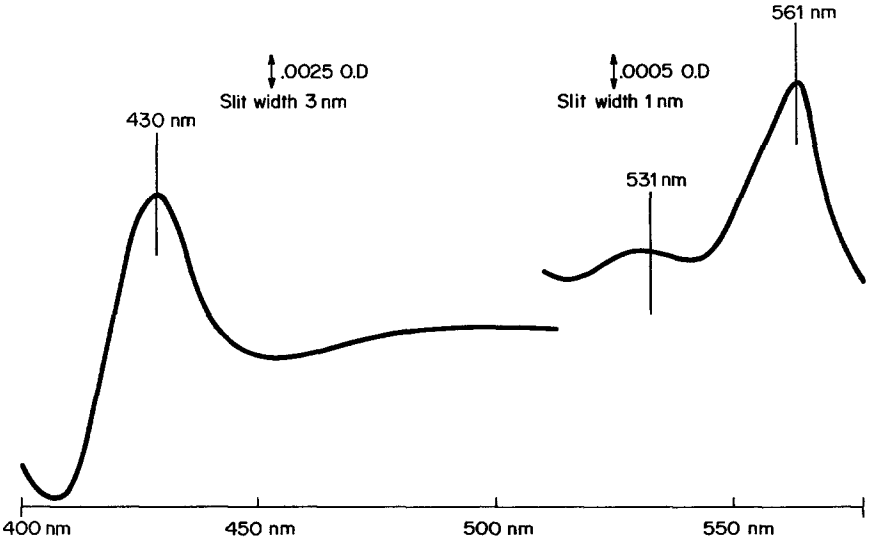

Fig. 1. Dithionite-reduced–oxidized difference spectrum of purified porcine erythrocyte plasma membranes recorded at room temperature. Membranes were suspended in 50% glycerol, 0.125 M sodium phosphate buffer with 5.5 mg membrane protein per milliliter.

Table III. Copper Content of Plasma Membranes Compared with Other Membranes

Membrane	Copper (nmol/mg protein)	Reference
Pig erythrocyte plasma membrane	0.45 ± 0.04 (3)	
Rat liver plasma membrane	2.1	Vassiletz *et al.* (1976)
Rat liver endoplasmic reticulum	2.0	Vassiletz *et al.* (1976)
Rabbit liver microsomes	0.38	Mason *et al.* (1965)
Beef heart mitochondria	2.4	Crane *et al.* (1956)
Rabbit liver endoplasmic reticulum	0.09	Ichikawa and Yamano (1970)
Rabbit liver Golgi membranes	0.21	Ichikawa and Yamano (1970)
Rabbit liver plasma membrane	0	Ichikawa and Yamano (1970)

cytochrome iron have been reported by Vassiletz *et al.* (1976). They find 5.7 nmol/mg protein total iron and 0.16 nmol cytochrome iron/mg protein in rat liver plasma membrane. The iron content of rat liver endoplasmic reticulum and Golgi membrane has been reported to be high or higher than the amounts we find in the mouse liver membranes. Vassiletz *et al.* (1976) found 10 nmol/mg protein (heme 1.4 nmol/mg protein) in endoplasmic reticulum. Morré *et al.* (1974) found 35 nmol/mg protein (heme 1.2 nmol/mg protein),

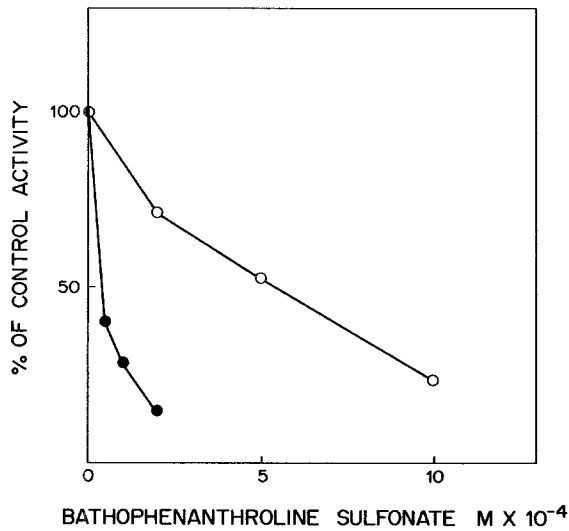

Fig. 2. Bathophenanthroline sulfonate inhibition of NADH ferricyanide reductase in porcine erythrocyte plasma membranes compared to effect on NADH cytochrome *c* reductase activity. (○) ferricyanide reductase; (●) cytochrome *c* reductase. Assay as described in Materials and Methods. Control activity NADH ferricyanide 279 and NADH cytochrome *c* 4.2 nmol/min/mg protein.

and Wills (1969) found 28.6 nmol/mg protein in rat liver endoplasmic reticulum. On the other hand, nonheme iron in rabbit liver microsomes has been reported at 3.3 nmol/mg protein (Mason *et al.*, 1965). Rat liver Golgi apparatus have 36.4 nmol Fe/mg protein and only 0.6 nmol heme/mg protein (Morré *et al.*, 1974).

The iron in fatty acid desaturase is unlikely to account for a large part of the nonheme iron since it would represent about 0.15 nmol/mg protein in rat liver microsomes (Strittmatter *et al.*, 1974). It should be noted, however, that bathophenanthroline sulfonate extracts the iron from the desaturase. Wills has shown that one-third of rat liver microsomal iron is extracted with bathophenanthroline sulfonate (Wills, 1969). The inhibition of the plasma membrane NADH ferricyanide reductase by bathophenanthroline sulfonate may indicate a similar type of iron binding in the dehydrogenase. It is clear from the failure of hydrochloric acid to release most of the plasma membrane iron that it is not in the form usually found in iron–sulfur protein (Orme-Johnson, 1973).

The reported copper content of the three plasma membrane preparations shows considerable variation. The copper found in both the rat liver plasma membrane and the pig erythrocyte membrane is not a part of cytochrome oxidase as in mitochondria. It is also not clear that it would be associated with an ascorbate oxidase since none of that activity is observed in erythrocyte membrane. It is clear that a further examination of copper distribution in different types of endomembranes is needed.

It appears that there is sufficient nonheme iron in plasma membranes to be significant in the redox function in these membranes. This iron may not be in the typical iron–sulfur type of structure and, therefore, may not give ESR signals common to that type of protein. An example of this type of iron is found in the fatty acid desaturase (Strittmatter *et al.*, 1974).

Acknowledgments

This work was supported by a grant from the National Institute of Arthritis, Metabolic, Digestive, and Kidney Diseases AM25235. F. L. Crane is the recipient of a Research Career Award from NIH K6-GM21839. We wish to thank Chris Jewell for technical assistance.

References

Beinert, H. (1978). *Methods Enzymol.* **54,** 435–445.

Bruder, G., Bretscher, A., Franke, W., and Jarasch, E. (1980). *Biochim. Biophys. Acta* **600,** 739–755.

Crane, F., Glenn, J., and Green, D. (1956). *Biochim. Biophys. Acta* **22,** 475–487.
Crane, F., Goldenberg, H., Morré, D., and Löw, H. (1979). In *Subcellular Biochemistry* (Roodyn, D. B., ed.), Plenum Press, New York, pp. 345–399.
Crane, F., MacKellar, W., Morré, D., Ramasarma, T., Goldenberg, G., Grebing, C., and Löw, H. (1980). *Biochem. Biophys. Res. Commun.* **93,** 746–754.
Dodge, J., Mitchell, C., and Hanahan, D. (1963). *Arch. Biochem. Biophys.* **100,** 119–130.
Giacobino, J., and Chmelar, M. (1977). *Biochim. Biophys. Acta* **487,** 269–276.
Goldenberg, H., Crane, F., and Morré, D. (1979). *J. Biol. Chem.* **254,** 2491–2498.
Ichikawa, Y., and Yamano, T. (1970). *Biochem. Biophys. Res. Commun.* **40,** 297–305.
Jarasch, E., Bruder, G., Keenan, T., and Franke, W. (1977). *J. Cell Biol.* **73,** 223–241.
Mason, H., North, J., and Vanneste, M. (1965). *Fed. Proc.* **24,** 1172–1180.
Morré, D., Cheethan, R., Nyquist, S., and Outraacht, L. (1972). *Biochemistry* **2,** 61–69.
Morré, D. (1973). In *Molecular Techniques and Approaches in Developmental Biology* (Crispeels, M. J., ed.), Wiley, New York, pp. 1–27.
Morré, D., Keenan, T., and Huang, C. (1974). *Adv. Cytopharmacol.* **2,** 107–125.
Orme-Johnson, W. (1973). *Annu. Rev. Biochem.* **42,** 159–204.
Ormstad, K., Moldens, P., and Orrenius, S. (1979). *Biochem. Biophys. Res. Commun.* **89,** 497–503.
Strittmatter, P., Spatz, L., Corcoran, D., Roger, M., Stelow, B., and Redline, R. (1974). *Proc. Natl. Acad. Sci. USA* **71,** 4565–4569.
Vassiletz, I., Derkatchev, E., and Neifakh, S. (1976). *Exp. Cell Res.* **46,** 419–427.
Wills, E. (1969). *Biochem. J.* **113,** 325–332.