INVESTIGATIONS ON THE RELEASE OF MEMBRANE-BOUND GLYCERAL-DEHYDE-3-PHOSPHATE DEHYDROGENASE

G. LETKO and R. BOHNENSACK
Physiologisch-chemisches Institut der Medizinischen Akademie Magdeburg, GDR

Received 3 December 1973

1. Introduction

There are many studies of the binding of the GAPDH* in erythrocytes to the cell membrane [1–7]. The question of GAPDH binding in vivo has not been solved [2–5, 8].

A detailed characterisation of the release of bound GAPDH does not exist.

The present paper describes investigations of the unspecific effects of ultrasonics, pH-changes, ionic strength and the specific effect of NAD on the association of the enzyme with the membrane.

2. Materials and methods

Reticulocyte-rich cell suspension (about 40% reticulocytes, 60% erythrocytes) prepared from fresh rabbit blood was hemolysed (dilution 1:100) with 10 mM TRA buffer (pH 7.2)+ and then centrifuged at 20 000 *g* (15 min). After washing in 10 mM HEPES (pH 7.2) the pellet was resuspended in the same buffer and used as 'bound GAPDH'.

In accordance with Shin and Carraway [7] after hemolysis about 60–70% of the total activity remained associated with the particulate fraction. The enzyme activity was determined according to Bergmeyer [9].

To study the release of the bound enzyme a certain amount of bound GAPDH was incubated for 5 min and then centrifuged at 20 000 *g*. Thereupon the enzyme activity was assayed in the pellet and the supernatant.

3. Results

Repeated washings of the bound GAPDH with 10 mM HEPES (pH 7.2) demonstrate the stability of the GAPDH binding to the membrane (fig. 1). The portion of the bound enzyme does not decrease, whereas the specific activity of the GAPDH increases as a result of the release of inactive proteins.

Fig. 2 represents the results of the treatment of bound GAPDH with buffer solutions of different pH.

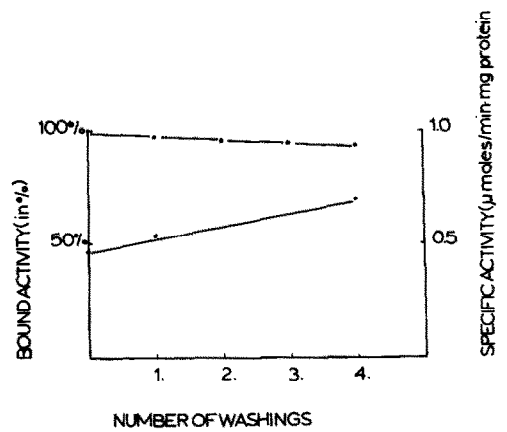

Fig. 1. Repeated washings of membrane-bound GAPDH with 10 mM HEPES (pH 7.2): (●) bound enzyme in percent of the total activity; (○) specific activity in μmoles/min per mg protein.

* *Abbreviations*: 1,3 DPG – 1,3-diphosphoglyceric acid; GAPDH – glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12); GAP – glyceraldehyde-3-phosphate; HEPES – *N*-2-hydroxyethylpiperacine-*N*-ethansulfonic acid; NSA – nicotinamide; TRA – triethanolamine.

+ All buffers used contained 1 mM mercaptoethanol.

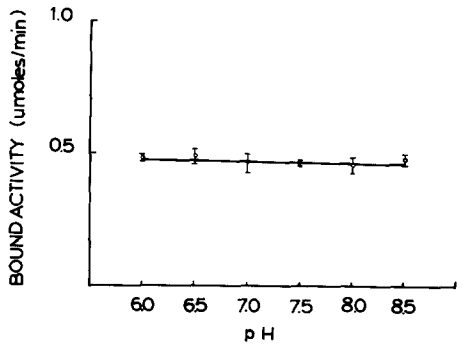

Fig. 2. Treatment of bound GAPDH with 10 mM Good [10] buffers of different pH.

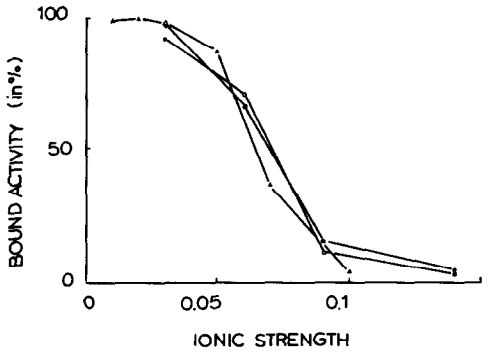

Fig. 3. Influence of electrolytes of different ionic strength on the GAPDH–membrane binding. Bound GAPDH was incubated for 5 min in 10 mM HEPES medium, pH 7.2 containing: KCl (△—△—△); $Mg(CH_3COO)_2$ (●—●—●) or K_2SO_4 (○—○—○) with increasing ionic strength.

Under experimental conditions changing of pH did not cause a release of bound GAPDH. As the results in table 1 show, the binding of GAPDH to the membrane is practically not destroyed by ultrasonics. Fig. 3 represents the influence of different electrolytes on the release of bound GAPDH. This release does not depend on the species of the electrolyte but on the ionic strength of the solution. This suggests that there is no specific interaction between these ions and the enzyme and the membrane, respectively. Up to an ionic strength of 0.04 a release of the bound enzyme does not take place, whereas in the range from 0.05 to 0.1 a straight dependence of enzyme release on the ionic strength exists.

Further experiments show that besides the unspecific release of the bound enzyme by electrolytes of certain ionic strength a specific release exists caused by NAD.

The small NAD concentrations, which are already active, exclude an unspecific ionic strength effect of the NAD. The NAD influence on the enzyme–membrane binding is strongly dependent on the pH of the incubation medium (table 3). Whereas below pH 7.5, 1 mM NAD does not cause an enzyme release, above pH 8.2 GAPDH is totally released by this NAD concentration.

0.1 mM NAD, NADH, AMP or NSA do not influence the GAPDH–membrane binding at pH 7.5 (not represented results). As shown in fig. 3, an electrolyte solution of an ionic strength of 20 mM does not release bound GAPDH too. But a combination of NAD, NADH or AMP with an electrolyte solution (e.g. 6.7 mM $Mg(CH_3COO)_2$) causes a strong enzyme release (table 4).

Table 1
Release of bound GAPDH by ultrasonication.

Sonication time [sec]	GAPDH activity in the pellet [μmoles/min]	GAPDH activity in the supernatant [μmoles/min]	Released GAPDH in % of the total
0	0.56	0.008	1
1 × 10	0.66	0.028	4
2 × 10	0.54	0.032	6
3 × 10	0.48	0.020	4
9 × 10	0.46	0.040	8

Bound GAPDH was suspended in 4 ml 10 mM HEPES (pH 7.5) and sonicated at 0°C in 10 sec intervals with an MSE ultrasonic desintegrator (amplitude 6 μm). After centrifugation (1 hr; 110 000 *g*) the enzyme activity was determined in pellet and supernatant.

Table 2
Dependence of the release of bound GAPDH on NAD in 10 mM Tris, pH 8.2.

NAD [mM]	GAPDH activity in the pellet [μmoles/min]	GAPDH activity in the supernatant [μmoles/min]	% released GAPDH
1.2	0	0.18	100
0.12	0.07	0.12	63
0.06	0.09	0.1	53
0.024	0.1	0.09	47
0.012	0.14	0.05	36
0.0012	0.18	0.01	5

Table 3
The pH influence of the incubation medium on the releasing effect of 1 mM NAD to bound GAPDH in 10 mM Good [10] buffers.

pH	GAPDH activity in the pellet [μmoles/min]	GAPDH activity in the supernatant [μmoles/min]	% released GAPDH
7.5	0.14	0	0
8.0	0.07	0.15	32
8.2	0.01	0.18	95
8.5	0.01	0.21	95
9.0	0.01	0.15	94

This observed sensitization of NAD, NADH, and AMP effect does not exist in the case of NSA.

4. Discussion

The GAPDH is – as shown in the washing experiments and by ultrasonication – tightly bound to the membrane of erythrocytes and reticulocytes, respectively. The sensibility of the GAPDH–membrane binding to electrolytes with an ionic strength greater than 0.04 makes us assume electrostatic interactions between enzyme and membrane as already reported by other authors [3,7].

Additionally the enzyme binding is influenced by metabolites. Shin and Caraway [7] supposed that the observed release of GAPDH during hemolysis of erythrocytes by ATP is caused by an alteration of the membrane by ATP, which is then unable to bind the enzyme. By reason of specific interaction between NAD and the GAPDH molecule [11,12] it is assumed that the releasing effect of NAD on membrane-bound GAPDH is caused by such interaction, too, and not by an interaction with the membrane.

As shown in table 4, the cooperativity between

Table 4
Release of bound GAPDH by combination of ionic strength effect and a specific effector.

Effector	GAPDH activity in the pellet [μmoles/min]	GAPDH activity in the supernatant [μmoles/min]	% released GAPDH
NAD	0.02	0.43	96
NADH	0.02	0.47	96
AMP	0.08	0.27	77
NSA	0.31	0	0

The incubation medium contained: 10 mM HEPES (pH 7.5), 6.7 mM $Mg(CH_3COO)_2$ and 0.1 mM effectors.

NAD and ionic strength effect produces a sensitization of the NAD effect. This explains the impossibility of measuring the activity of the bound GAPDH [13]. Probably membrane binding and effector binding to the enzyme exclude one another.

If the GAPDH membrane association is influenced by effectors in vivo too, an alteration of the free metabolite concentration can be caused by changing the bound GAPDH portion. In this way via free NAD or NADH concentration the metabolite fluxes can be influenced.

Acknowledgements

The authors wish to thank Mrs. K. Frank and Mrs. G. Sackewitz for their careful technical assistance.

References

[1] Schrier, S.L. (1963) J. Clin. Invest. 42, 756.
[2] Green, D.E., Murer, E., Hultin, H.O., Richardson, S.H., Salmon, B., Brierley, G.P. and Baum, H. (1965) Arch. Biochem. Biophys. 112, 635.
[3] Mitchell, C.D., Mitchell, W.B. and Hanahan, D.J. (1965) Biochim. Biophys. Acta 104, 348.
[4] Ronquist, G. (1969) Acta Physiol. Scand. 76, 312.
[5] Tanner, M.J.A. and Gray, W.R. (1971) Biochem. J. 125, 1109.
[6] Carraway, K.L. and Shin, B.C. (1972) J. Biol. Chem. 247, 2102.
[7] Shin, B.C. and Carraway, K.L. (1973) J. Biol. Chem. 248, 1436.
[8] Nilsson, O. and Ronquist, G. (1969) Biochim. Biophys. Acta 183, 1.
[9] Bergmeyer, H.U. (1970) Methoden der enzymatichen Analyse, Akademie Verlag, Berlin, 2nd ed., p. 425.
[10] Good, M.E. et al. (1966) Biochemistry 5, 467.
[11] Eby, D. and Kirtley, M.E. (1971) Biochemistry 10, 2677.
[12] Ellenrieder, G. von, Kirschner, K. and Schuster, L. (1972) Eur. J. Biochem. 26, 220.
[13] Letko, G., Bohnensack, R. and Augustin, W. (1973) VIIth International Berlin Symposium on Structure and Function of erythrocytes.