Volume 227, number 1, 32–34 FEB 05486 January 1988

The turnover number for band 3-mediated sulfate transport in phosphatidylcholine bilayers

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Received 16 November 1987

The anion transport system of the human erythrocyte membrane was reconstituted in unilamellar phosphatidylcholine vesicles, and a vesicle subpopulation of a narrow size distribution was isolated from the sample by gel filtration. In this subpopulation, the turnover number of the transport protein (the band 3 protein) for sulfate transport was determined. It was found that, in the reconstituted system, the protein transports sulfate 5–10-times faster than in the human erythrocyte membrane.

Band 3 protein; Sulfate transport; Reconstitution; Turnover number; (Erythrocyte membrane)

1. INTRODUCTION

The most prominent transport function of the human erythrocyte membrane is the exchange of anions between the interior and exterior of the cell. It is mediated by 'band 3', the membrane's most abundant protein which is present in 0.8–1.2 million copies per cell [1,2]. The anions transported in vivo are Cl^- and HCO_3^-; their exchange has a halftime <0.1 s at 37°C [2]. However, most transport studies on this system follow the exchange of sulfate, which is slower than that of the monovalent anions by several orders of magnitude and can therefore be measured more conveniently. The present paper deals with the transport capacity of the band 3 protein for sulfate.

At 37°C, pH 7.2 and a sulfate concentration of 10 mM, one band 3 molecule in the intact human erythrocyte membrane can exchange approx. 85 sulfate ions per minute (as calculated from the data in [3,4]). There are, however, indications that this figure may not represent the highest rate at which sulfate can be transported by band 3 [5,6]. We will show in the following that, in fact, band 3 reconstituted in phosphatidylcholine vesicles can transport sulfate anions approx. 5–10-times faster than in the human erythrocyte membrane.

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Abbreviations: PC, phosphatidylcholine; H_2DIDS, 4,4′-diisothiocyanostilbene-2,2′-disulfonate

2. MATERIALS AND METHODS

2.1. *Reconstitution procedure*

The incorporation of ^{3}H-labelled band 3 protein into bilayer membranes of egg phosphatidylcholine (PC) and the subsequent isolation of the band 3-PC vesicles were performed as in [7], with the modifications described in [8] which lead to an increased trapped volume of the vesicle samples and to vesicle subpopulations of a narrower size distribution. The protein/lipid ratio in the starting mixture was around 1 : 50 (w/w).

2.2. *Transport studies*

For the measurement and evaluation of the sulfate efflux from the vesicles see [7]. The buffer used during the flux measurements was 10 mM Hepes (pH 7.2), 10 mM Na_2SO_4, 0.5 mM EDTA, and temperature was 37°C. Turnover number, τ, for band 3-mediated sulfate transport was calculated from the rate constant, k_1, of the inhibitor-sensitive component of the sulfate efflux, the specific internal volume, v_i, of the vesicles (in liters per mol of phospholipid), and the molar protein/lipid ratio α in the vesicle membranes by applying eqn 1 (which can be easily verified) [7]:

$$\tau = \frac{k_1 \cdot c \cdot v_i}{\alpha} \qquad (1)$$

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

Different from [7,8], v_i was calculated from the average vesicle diameters, as obtained by electron microscopy of freeze-dried samples (see below) [9]. In this method of determining τ, it is assumed (i) that all vesicles are of identical size, and (ii) that those vesicles which are so leaky for sulfate that they do not contribute to the flux measurements [7,8] have the same (average) protein/lipid ratio as the sealed vesicles.

2.3. *Electron microscopy*

The vesicles were visualized in a Philips 300 electron microscope. The samples were prepared either by negative staining [7] or by freezing in liquid propane, followed by freeze-drying in an EPA 100 apparatus (Leybold-Heraeus, Köln, FRG) and shadowing with platinum/carbon.

3. RESULTS

The reconstituted anion transport system in unilamellar PC vesicles applied in this paper reproduces all major properties of the sulfate transport across the human erythrocyte membrane [7]. The vesicle population obtained according to [7] is heterogeneous in size, however, vesicles of a narrow size distribution can be isolated from it by gel filtration on Sephacryl S-1000 [8]. An electron micrograph of these vesicles is shown in fig.1. The average vesicle diameter derived from this and other figures is 125–135 nm, in agreement with our previous data from dynamic light scattering measurements [8]. The relative width of the size distributions, as characterized by the standard deviation, is around ± 10% [8] and thus small enough to warrant application of eqn 1 for the determination of τ and calculation of v_i from the average vesicle diameter. Using the data in [9], we obtained v_i = 4.0 l per mol of phospholipid.

To assess the validity of assumption (ii) (see section 2.2), we have determined the protein/lipid ratio of different fractions from the density gradient which served for separating the protein/lipid vesicles from mixed Triton/PC/band 3 micelles [7]. When the vesicles are formed in the presence of 10% sucrose, the dominating factor in the separation of the vesicles is whether or not they are

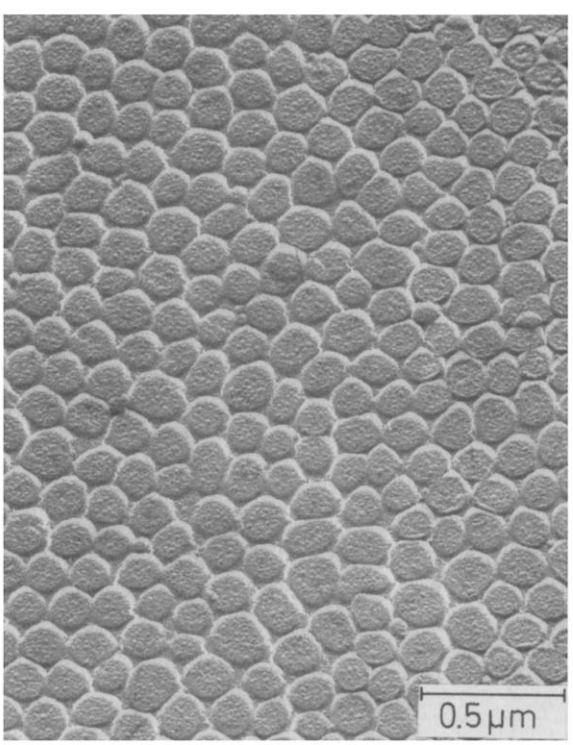

Fig.1. Electron micrograph of the band 3/PC vesicles after reducing the width of the size distribution by gel filtration. The samples were prepared by freeze-drying/Pt-C shadowing.

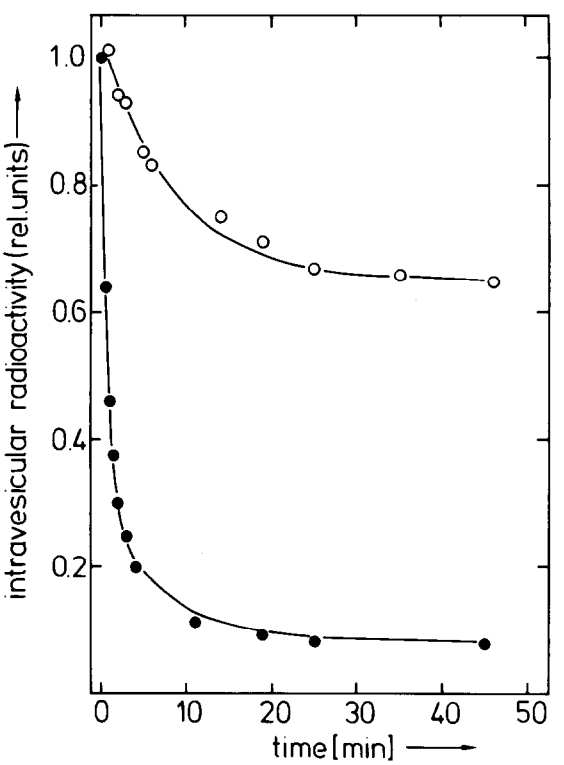

Fig.2. Sulfate efflux from vesicles of a narrow size distribution, in the absence of inhibitors of anion transport (●) and after addition (at zero time) of 15 µM H_2DIDS (○). The curves were fitted to the data according to [7].

Table 1

Values of k_1, α and τ obtained with 5 different vesicle preparations

Prep. no.	k_1 (min^{-1})	α	τ (min^{-1})
1	0.455	3.0×10^{-5}	603
2	0.297	2.7×10^{-5}	437
3	0.733	6.1×10^{-5}	481
4	1.20	4.8×10^{-5}	1000
5	1.13	10.6×10^{-5}	426

T = 37°C. Each value of k_1 is the mean from two flux measurements

sealed for sucrose [8]. It was found that the different fractions had virtually the same protein/lipid ratio. Thus, sealed and leaky vesicles do not differ in their average protein content.

The sulfate efflux from the vesicle subpopulation isolated by gel filtration was found to be very similar to that from the unfractionated samples. An efflux curve is shown in fig.2. Analysis of the curves according to [7] yields the rate constant k_1 of the specific (inhibitor-sensitive) flux component. The values of k_1 at 37°C, pH 7.2 and a sulfate concentration of 10 mM, together with the corresponding values of the protein/lipid ratio α in the vesicle membranes and of the turnover number τ calculated by means of eqn 1 are shown in table 1 (for data from 5 different preparations). From the individual τ values, an average turnover number of approx. 590 min^{-1} is obtained. We estimate that the error of this figure does not exceed ± 50%. As stated in the introduction, the turnover number of band 3 for sulfate transport in the human erythrocyte membrane, under the same experimental conditions, is 85 min^{-1}.

4. DISCUSSION

We have shown in this paper that the anion transport system of the human erythrocyte membrane, when reconstituted in PC bilayers, can transport sulfate 5–10-times faster than the same system in the intact membrane. It thus seems that, in the human erythrocyte membrane, only a relatively small part of the system's sulfate transport capacity is available.

What could be the reasons for the apparent increase in the turnover number for sulfate transport in the reconstituted system, as compared to the native one? One reason could be the difference in the lipid composition of the two systems. This idea is supported by the observations in [5,6]. Another reason could be the difference in the state of association of the transport protein, band 3: in the human erythrocyte membrane, the most frequently occurring band 3 aggregates probably are the dimers and tetramers [10,11], whereas in the vesicles, with their low protein/lipid ratio, the association equilibrium of band 3 [10–12] should be shifted towards the monomer. The reconstituted anion transport system applied by us may be a useful tool to study these questions.

Acknowledgements: We are grateful to Professor H. Passow for stimulating discussions and to the Deutsche Forschungsgemeinschaft for financial support (SFB 169).

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