Demonstration of Two ATPases in Human Erythrocyte Membranes

In 1960 POST et al.[1] described an ATPase activity in preparations of erythrocyte membranes which is involved in the active transport of monovalent cations. Varying reports have been published since on the sensitivity of red cell membrane ATPase to -SH reagents. While organic mercurials (e.g. Salyrgan) were found to inhibit the enzymatic activity at 50% with a concentration of 0.15 mM[2], apparently no inhibition could be observed of N-ethylmaleimid (NEM) on the ATPase of human erythrocytes[3]. In contrast, TOSTESON[4] found that NEM readily blocks the Na^+, K^+-sensitive ATPase of sheep red cell membranes, but not the Na^+, K^+-insensitive enzyme, thus resembling the action of cardiac glycosides. The data presented here indicate a similar differentiation of ATPases in human erythrocyte membranes, with respect to impairment by NEM.

Hemoglobin-free red cell membrane fragments were prepared according to POST[1]. They contained, as shown by amperometric titration[5], an average of 0.046 μM-SH/mg protein (range 0.035–0.061 $n = 12$) and liberated in the assay system of NAKAO[6] 1.07 μM PO_4^{---}/mg protein (range 0.57–1.67, $n = 12$). The membrane fragments were incubated with several concentrations of NEM (purified by sublimation) in 0.04 M *Tris*-HCl buffer pH 7.4 at 4 °C and the action of NEM was controlled over a period of 2 h by -SH titration and ATPase assay of samples taken simultaneously. SH titrations were carried out within 5 min after sampling. An excess of cysteine was added to the ATPase assay system to block further action of NEM.

The inactivation of membrane ATPase by NEM is swift and does not run parallel to the rate of -SH blockade (Figure 1, a and b). At a concentration of 0.5 μM NEM/μM titratable -SH, the enzymatic activity decreases within 10 min of incubation to the 50% level, reaching a loss of 72% after 120 min. The corresponding blockade of -SH groups amounts to 15% and 22% respectively. Although two- and fourfold excess of NEM finally leave only about 20% of the titratable -SH groups of the membrane fragments unreacted, no substantial further effect on the ATPase is elicted by the higher NEM concentrations.

Two possible interpretations of these data are: (1) The enzymatic activity is confined to one molecule. 20% of this enzyme is not accessible for NEM. (2) The membrane ATPase activity is represented by 2 individual enzymes, only one of which is blocked by NEM. In a first attempt to rule out one of the two possibilities, membrane fragments were subjected to tryptic digestion in an autotitrator. No measurable hydrolysis could be detected, but all ATPase activity was lost within a few minutes after the addition of trypsin (Figure 2). Since it is difficult to assess how the bulky trypsin molecule could have better access to the membrane ATPase than the low molecular NEM, the second possibility appears to be the more feasible one. The trypsin effect, furthermore, points to a surface-localization of the 2 enzymes, which can be either at the outer or the inner surface of the membrane or at both (see also[7]). Isolation of the enzymes

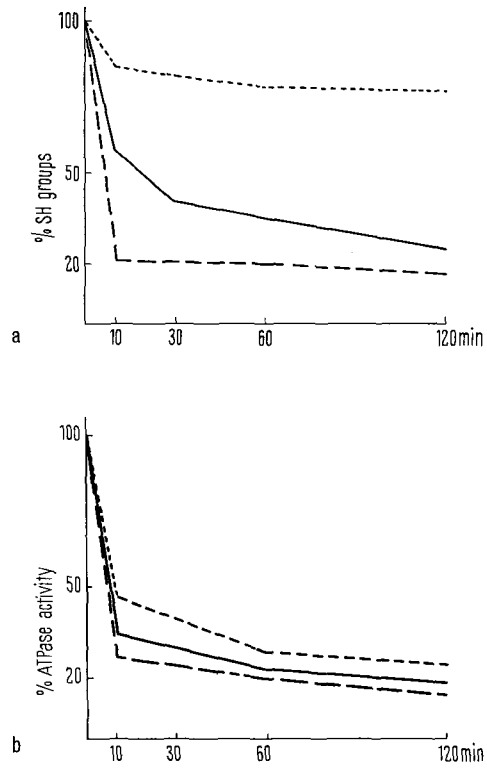

Fig. 1. Membrane ATPase incubated with 0.5 (---), 2 (—), 4 (---) μM NEM/μM titratable -SH groups for 120 min; (a) decrease of titratable -SH groups, (b) decrease of ATPase activity, in per cent of initial values.

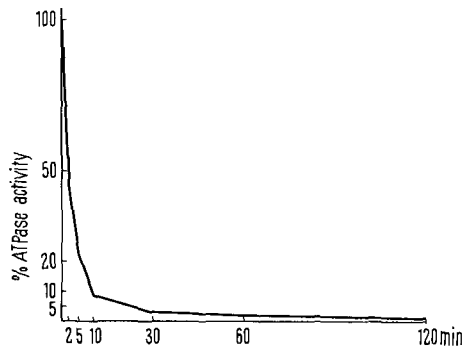

Fig. 2. Membrane ATPase (6 mg/ml), incubated with 2% trypsin; at indicated intervals 0.5 ml samples pipetted into 0.1 mg of trypsin inhibitor at 4 °C, ATPase measured at termination of experiment.

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and localization experiments, e.g. with immunological methods, could substantially aid the knowledge of membrane structure.

It has been suggested that the ATPase system in red cell membranes may be represented by an actomyosin-type protein[8,2]. In our hands, extracts of red cell membranes prepared by various procedures used for the isolation of contractile proteins from smooth and striated muscle[9,10] and from thrombocytes[11] contained no actomyosin-like protein neither on biochemical or on immunological analysis (including immunefluorescent studies).

Zusammenfassung. Menschliche Erythrozytenmembranen enthalten eine NEM-sensitive und eine NEM-insensitive ATPase, die wahrscheinlich beide an Membranoberflächen lokalisiert sind.

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Level of Ascorbic Acid and its Oxidation in the Liver of the Scorpion, *Palamnaeus bengalensis*

It is well known that in all vertebrates ascorbic acid is widely required in metabolism in addition to its role in the synthesis of collagen. Several workers have also reported the nutritional and metabolic status of ascorbic acid in invertebrates[1,2]. However, no information is available on the metabolism of ascorbic acid in arachnids. The concentration of total ascorbic acid in the liver of the scorpion, *Palamnaeus bengalensis*, and the rate of its oxidation in this organ is reported here.

Scorpions weighing approximately 5.0 g were collected locally and were used within 24 h of their collection. The liver was removed and immediately used for the estimation of ascorbic acid[3] using a Klett-Summerson colorimeter. The concentration of ascorbic acid in each sample was determined from a standard curve which was linear and was drawn each time. The average ascorbic acid concentration from the liver of 8 scorpions was found to be 5.567 ± 0.426 mg/100 g dry weight.

The rate of oxygen consumption of the liver homogenate with ascorbic acid and other substrates was studied manometrically. The temperature of measurement was 32 °C and the gas phase was air. The total volume of the reaction mixture after addition of the homogenate was 3.0 ml. The pH of the incubation mixture was maintained at 6.5 with phosphate buffer[4]. The centre well contained 0.2 ml of 20% KOH. Duplicate flasks were used for each experiment. The final concentrations of the various reagents of the reaction mixture were: sucrose 83.3; phosphate buffer 8.8; $MgCl_2$ 3.3; ascorbic acid 16.6; glucose, succinate and citrate 33.3 µmoles. Readings were taken at 10 min intervals for 60 min. The oxygen consumption was expressed as Q_{O_2} (ml O_2/g dry wt./h) after determining the dry weight of the tissue.

The level of ascorbic acid in the liver of scorpion is comparatively less than that of the rat (24.0 mg/100 ml) and frog (22.0 mg/100 ml)[5]. However, its concentration is as high as that of the fish (3.2–23.0 mg/100 ml)[5]. Similarly, the Q_{O_2} value with ascorbic acid is much higher than those obtained for other substrates (Table). The rates of oxidation of different substrates are: ascorbic acid > citrate > glucose > succinate.

The high concentration of ascorbic acid and its rapid oxidation in the liver of the adult scorpion is of particular interest since the collagen content of the animal is negligible. Therefore, it may be required for the 3 following functions: (1) the animal may require ascorbic acid for its metabolic activities as do rats[6]; (2) it may be an intermediate of the uronic acid pathway which is interlinked with the hexosemonophosphate pathway[7]; (3) it may be necessary for the metabolism of tyrosine as is reported for *Blatta conjuncta*[8]. The latter may be the major requirement for ascorbic acid since oxidation of tyrosine is needed for melanin synthesis of the cuticle as in the insects[9].

Q_{O_2} (ml O_2/g dry wt./h) of the liver homogenate of scorpion with various substrates

Substrates	Q_{O_2}
Ascorbic acid	0.42 ± 0.025 (4)
Glucose	0.27 ± 0.034 (5)
Succinate	0.15 ± 0.010 (5)
Citrate	0.40 ± 0.028 (5)

The figures in parentheses indicate the number of animals used.

Zusammenfassung. Kolorimetrische und manometrische Untersuchungen zur Konzentrationsbestimmung der Ascorbinsäure und ihrer Oxydationsrate in der Skorpionsleber bei *Palamaeus bengalensis*.

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