A C T A U N I V E R S I T A T I S L O D Z I E N S I S
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THE INFLUENCE OF ALLOXAN ON ERYTHROCYTE MEMBRANE PROTEINS

The interaction of alloxan with isolated erythrocyte membranes has been investigated by ESR in the 10-50°C temperature range. The results show that alloxan-treated membranes are less sensitive to the action of temperature compared with untreated samples.

INTRODUCTION

Alloxan is a chemical substance which produces selective damage to the pancreas. The selectivity of damage appears to depend upon a specific accumulation of alloxan in the pancreatic B-cells [5]. Other cells appear unaffected by injection of diabetogenic doses of alloxan into experimental animals [3].

The toxic action of alloxan is often attributed to the ability to undergo redox cycling reactions resulting in the production of active oxygen species leading to oxidative damage of cellular components [6, 9]. Alloxan is readily reduced to the alloxan radical and dialuric acid which spontaneously autoxidizes to form the superoxide radical, hydrogen peroxide and hydroxyl radical [14, 16, 21]. The site in the cell where alloxan generates free radicals is still unknown. Many have suggested it is the cell membrane [10, 15, 17].

In contrast to pancreatic B-cells which have been widely used as a model for alloxan action, very little is known about the effect of alloxan on other types of cells. The present study is devoted to the question whether alloxan in vitro perturbes the

structural integrity of erythrocyte membranes. We have investigated the interaction of alloxan with erythrocyte membrane proteins using the ESR technique.

MATERIALS AND METHODS

Pig erythrocyte membranes were prepared according to the method of D o d g e et al. [4]. The protein content was esti-

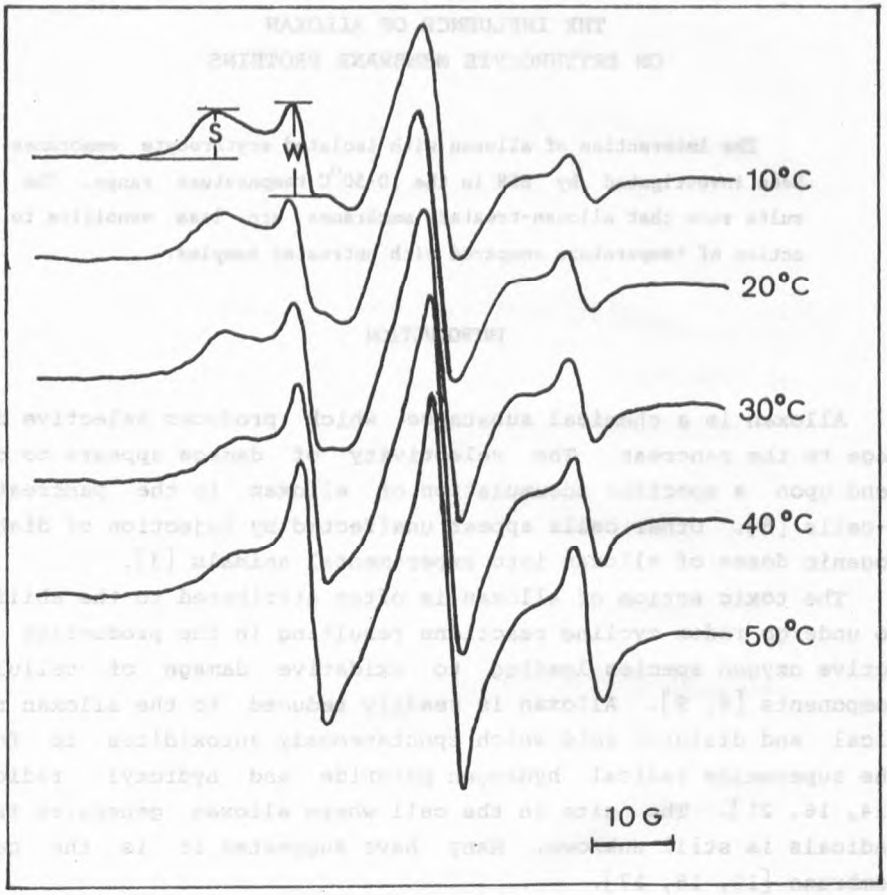

Fig. 1. ESR spectra of maleimide spin label bound to erythrocyte membranes. Sample of the labeled membranes was incubated with 1 mM alloxan for 30 min. ... 20°C and then the spectra were progressively recorded at indicated temperatures

mated by the method of L o w r y et al. [13]. The membranes suspended in phosphate buffer (pH 7.4) reacted overnight at 4°C with maleimide spin labels dissolved in ethanol. The membranes were incubated with maleimide in a weight ratio of 1 mg of maleimide to 25 mg of protein [2]. After being washed with phosphate buffer (pH 7.4) maleimide spin labeled membranes were incubated with freshly prepared solution of alloxan in phosphate buffer for 30 min. at room temperature. Maleimide (4-maleimide--2,2,6,6-tetramethylpiperidinooxyl) and alloxan were purchased from Sigma.

The ESR spectra were recorded on a Varian E-109 B spectrometer with a variable temperature control unit. The spectra were recorded at 20-62.5 mW microwave power, 10 x 10^2 receiver gain, 100 kHz modulation frequency, 2-4 mT modulation amplitude, 0.5 s time constant, 4 min. scan time and 3390 field set. The ratio of the peak amplitude of the low field transitions for the weakly (W) and strongly (S) immobilized nitroxides has been used as an indicator of changes in protein conformation and environment within pig membranes (Fig. 1) [2]. The effect of alloxan on the ESR spectra of membrane proteins labeled with maleimide spin label has been investigated in the range of 10-50°C.

RESULTS AND DISCUSSION

The reaction of maleimide with membrane-associated proteins results in the generation of two dominant classes of bound spin labels. The two classes of spectral components, S and W, denote strongly and weakly immobilized label motions in the protein molecules in membranes [7]. Studies of many workers show that the W/S ratio is very sensitive to the physical state of the membrane. The experimental parameters, such as temperature, pH, ionic strenght and chemical agents will cause large changes in the W/S value [8, 12, 18].

Applying the procedures outlined by Butterfield and Markesbery [2] we have obtained for control erythrocyte membranes a value of 5.86 $\pm$0.47 for the W/S ratio using 4-maleimido-2,2,6,6-tetramethylpiperidinooxyl in 5 mM phosphate buffer, pH 7.4 at 20°C.

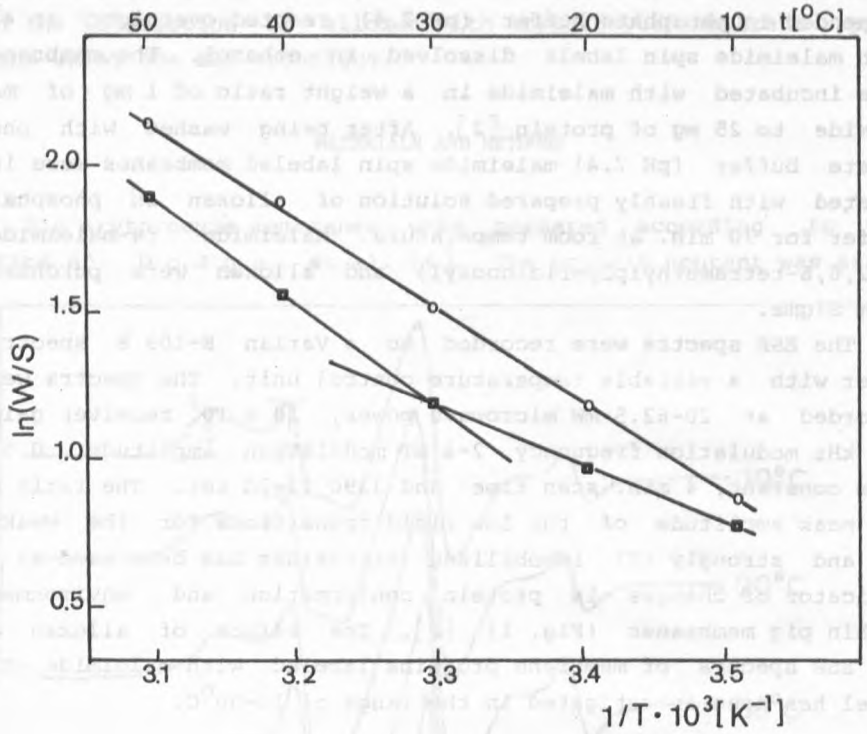

Fig. 2. The Arrhenius plot of ln W/S against reciprocal temperature for maleimide-labeled membranes. Samples were prepared and spectra were recorded as described in Fig. 1. ooo - control membranes, □□□ - 1 mM alloxan-treated membranes. These data represent the average of four different experiments

The ESR spectra of control and alloxan-treated membranes were recorded at different temperatures. (Fig. 1). Samples of maleimide-labeled membranes were incubated with 1 mM alloxan for 30 min. at 20°C and then after lowering the temperature of the sample to 10°C the spectra were progressively recorded at the indicated temperatures. Control spectra without alloxan were taken at the same conditions. The results indicate that the W/S ratio increased as the applied temperature was raised.

Addition of alloxan to isolated spin labeled membranes was found to perturb the W/S ratio as shown in Fig. 1 and 2. The W/S

ratio of alloxan-treated membranes changed from 2.13 at 10°C to 6.59 at 50°C, while untreated membranes showed a corresponding change from 2.39 to 8.64. Values of W/S ratio for control and 1 mM alloxan-treated membranes at 20°C are 3.30 and 2.60, respectively.

The Arrhenius plot of these results is given in Fig. 2. The values of W/S of the control membranes yield a straight line for temperatures 10-50°C. The plot of W/S of the 1 mM alloxan-treated samples however shows a remarkable discontinuity at 30°C. The data suggest that membranes incubated with alloxan are less sensitive to temperature action than untreated samples.

Fig. 3 shows the effect of alloxan concentration on the W/S ratio of maleimide-labeled membrane proteins. These samples were

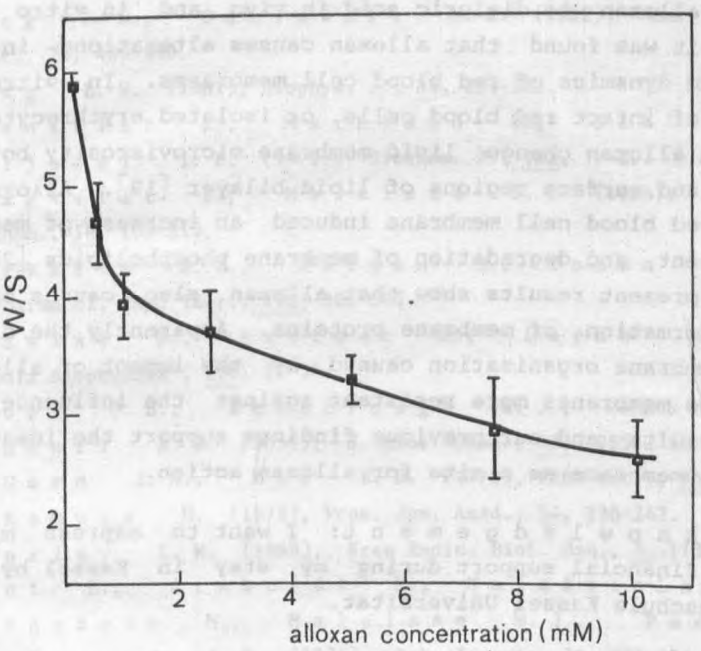

Fig. 3. Effect of alloxan concentration on the W/S ratio of maleimide-labeled membranes. Samples of the labeled membranes were incubated for 30 min. at 20°C with varying concentrations of alloxan (0.5-10 mM). ESR spectra were measured at 20°C. Each point represents the average ±SD of at least four different experiments

treated with 0.5-10 mM alloxan for 30 min. at 20°C and the spectra were recorded at the same temperature. The values of W/S decreased gradually with increasing alloxan concentration. Under these conditions the W/S ratio for 1 mM alloxan-treated membranes attain value of 3.91. The discrepancy between the W/S ratio at 20°C for both sets of data seems to indicate that upon lowering the temperature of the sample some irreversible change of membrane protein structure takes place and that the original state of membrane organization is not recovered after reheating to 20°C [1].

Alloxan is reactive drug which is reduced to dialuric acid. The autooxidation of dialuric acid yield active oxygen species [21]. The detection of hydrogen peroxide in erythrocytes after injection of alloxan suggests that cell-reducing agents similarly convert alloxan to dialuric acid in vivo and in vitro [11]. Recently, it was found that alloxan causes alterations in composition and dynamics of red blood cell membranes. In vitro incubation of intact red blood cells or isolated erythrocyte membranes with alloxan change lipid membrane microviscosity both in the deeper and surface regions of lipid bilayer [19]. Alloxan penetrate red blood cell membrane induced an increase of methemoglobin content and degradation of membrane phospholipids [20].

The present results show that alloxan also causes changes in the conformation of membrane proteins. Apparently the disturbance in membrane organization caused by the impact of alloxan renders the membranes more resistant against the influence of heat. These results and our previous findings support the idea pointing the cell membrane as a site for alloxan action.

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WPŁYW ALLOKSANU NA BIAŁKA BŁON ERYTROCYTÓW

Metodą ESR badano oddziaływanie alloksanu na wyizolowane błony erytrocytów w zakresie temperatur 10-50°C. Wyniki wskazują, że błony po inkubacji z alloksanem są mniej wrażliwe na działanie temperatury aniżeli preparaty kontrolne.