Thermal Lability of Membrane Proteins of Age Separated Erythrocytes as Studied by Electron Spin Resonance Spin Label Technique

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Z. Naturforsch. **42c,** 1343–1344 (1987); received March 13/October 16, 1987

Membrane Proteins, Erythrocyte Aging, Electron Spin Resonance, Spin Label Technique

Thermal lability of bovine erythrocyte membrane proteins was studied by electron spin resonance using maleimide spin label. The temperature of the sample during measurements could be varied for the first time between 0 and 60 °C with an accuracy of ± 0.1 °C. Our results show that "old" erythrocyte membrane proteins are less stable against thermal denaturation then "young" cells.

Introduction

The molecular mechanisms underlying the aging of red blood cells are still not understood. During their lifetime red cell proteins undergo a variety of modifications as racemization, glycosylation, deamidation, methylation and proteolysis [1, 2]. These processes may be the reason for the decreasing functional capacity of the cells and the thermal lability of cell proteins. Increasing thermal lability of several erythrocyte enzymes has been reported earlier [3].

The aim of the present study was to investigate the thermal stability of membrane proteins during erythrocyte aging by the spin label technique.

Materials and Methods

Bovine blood was obtained in an abbatoir and anticoagulated with Tri-sodium-citrate. The erythrocytes were separated according to density by the method of Murphy [4]. It has been demonstrated, that this method yields fractions of bovine erythrocytes of various age correlating with density [5]. Top 20% and bottom 20% of the cells were taken as representative of "young" and "old" red cell fractions respectively. Erythrocyte membranes were isolated according to the method of Dodge *et al.* [6]. The spin label 4-(N-maleimide)-2,2,6,6-tetramethyl-piperidine-1-oxyl (Mal-6) was obtained from Sigma. The membranes were labeled and studied in 10 mM sodium-phosphate buffer pH 7.4.

The electron spin resonance (ESR) spectra were taken in a Varian Modell E-109 B spectrometer at 9.5 GHz. The spectrometer was coupled to an Analog Devices Macsym 150 PC in order to register and analyze the data. The temperature dependence of the ESR signals was measured by means of a temperature control unit developed in this laboratory. With this equipment we were able for the first time to vary the temperature of the samples during taking the ESR spectra between 0 and 60 °C with an accuracy of ± 0.1 °C.

Results and Discussion

Mal-6 spin label bound to erythrocyte membranes yields complex ESR spectra due to the superposition of signals coming from weakly immobilized and strongly immobilized spin label residues (Fig. 1). The ratio of low field peak heights corresponding to weakly immobilized (hw) and strongly immobilized (hs) spin label residues is a convenient measure of the conformation of membrane proteins. Exposure of erythrocyte membranes to elevated temperatures

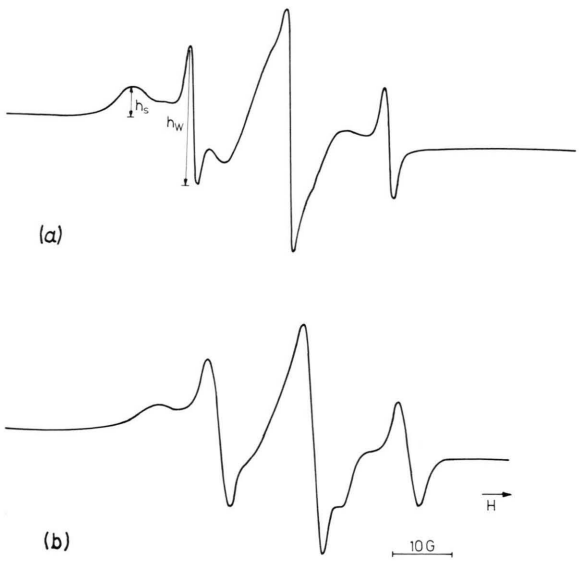

Fig. 1. ESR-spectrum of Mal-6 spin label bound to bovine erythrocyte membrane protein taken at 10 GHz. (a) room temperature, (b) T = 50 °C.

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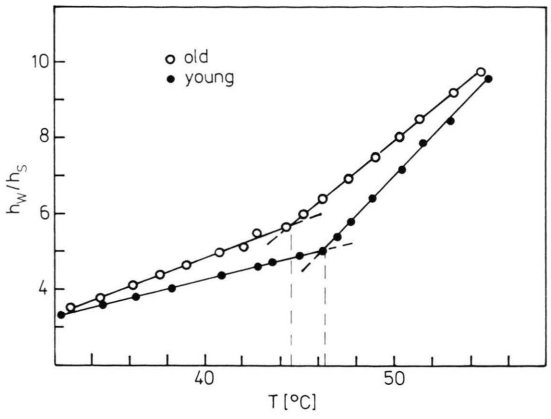

Fig. 2. Temperature Dependence of the ratio of peak heights of weakly immobilized to strongly immobilized spin labels for membranes of "young" and "old" erythrocytes.

Table I. Characteristic temperatures for the thermal denaturation of membrane proteins of "young" and "old" erythrocytes.

Exp. Nr.	Erythrocytes "young"	"old"
1	46.0	45.3
2	45.2	42.2
3	46.3	44.8
4	47.8	45.5
5	46.3	45.1
Mean value	46.5	44.6
S.D.	± 0.9	± 1.2
statistical significance (students' test)	$p < 0.025$	

brings about an increase of this ratio [7]. As shown in Fig. 2 we observed a change of slope of the hw/hs ratio *versus* temperature at a characteristic temperature. For membranes of non-fractionated erythrocytes this temperature is about 45 °C. Membranes of "young" and "old" erythrocytes had different characteristic temperatures. The difference amounting to about 2 °C (Table I).

In a previous study [7] the membranes were exposed to elevated temperatures but the ESR spectra were taken at room temperature. Under these conditions no characteristic temperature for the hw/hs ratio was observed (see Fig. 2 in [7]), although other parameters of the erythrocyte membranes showed discontinuities at about 45 °C.

The characteristic temperature observed in the present study apparently reflects a critical point at which thermally induced destabilization of erythrocyte membrane proteins occures. From our results it may be concluded that "old" erythrocyte membrane proteins are more vulnerable by thermal denaturation than "young" cells. This may be due to their greater conformational lability.

Acknowledgements

One of us (G.B.) wants to express his thanks for the financial support during his stay by the Gesamthochschule Kassel.

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