DECREASE IN 2,2,6,6-TETRAMETHYL-PIPERIDINE-1-OXYL (TEMPO) EPR SIGNAL IN PEROXYNITRITE-TREATED ERYTHROCYTE MEMBRANES

ANNA WROBEL
Institute of Physics, Wroclaw University of Technology, Wybrzeze Wyspianskiego 27, 50-370 Wroclaw, Poland

Treatment of erythrocyte membranes with peroxynitrite ($ONOO^-$), a cytotoxic species formed in vivo by the near diffusion controlled reaction of nitric oxide ($NO^\bullet$) and superoxide anion ($O_2^{-\bullet}$), led to loss of EPR signal of nitroxide radical 2,2,6,6-tetramethyl-piperidine-1-oxyl (TEMPO). Decrease in TEMPO EPR signal was peroxynitrite concentration dependent in studied peroxynitrite concentration range (100–1000 μM). An absence of such a phenomenon in control membranes (not treated with peroxynitrite) and in buffer treated with peroxynitrite indicates that the effect must be caused by reaction of nitroxide radicals with products of peroxynitrite reactions with membrane components. The same phenomenon was also studied in simple model systems (protein and lipid suspensions) and was observed both in lipid and protein systems treated by peroxynitrite, but in protein solution effect was bigger and occurred for lower peroxynitrite concentration. The effect of loss of EPR signal was significant both for erythrocyte membranes and bovine serum albumin (BSA) solution for peroxynitrite concentration of 100 μM, while in the case of linolenic acid suspension significant difference between control and peroxynitrite-treated sample was achieved for peroxynitrite concentration of 1000 μM. Comparison of the results obtained in lipid and protein systems suggests that the main role in the observed decrease in TEMPO EPR signal in peroxynitrite treated erythrocyte membranes is played by reaction of nitroxide radicals with protein derived species. This observation might be valuable for better understanding of interaction between nitroxides and peroxynitrite derived species in biological systems and might help in the design of rational therapies for pharmacological intervention using nitroxides as antioxidants.