Glycation and glycoxidation of proteins and peptides have been intensively studied and are considered as reliable diagnostic biomarkers of hyperglycemia and early stages of type II diabetes. However, glucose can also react with primary amino groups present in other cellular components, such as aminophospholipids (aminoPLs). Although it is proposed that glycated aminoPLs can induce many cellular responses and contribute to the development and progression of diabetes, the routes of their formation and their biological roles are only partially revealed. The same is true for the influence of glucose-derived modifications on the biophysical properties of PLs. Here we studied structural, signaling, and biophysical properties of glycated and glycoxidized phosphatidylethanolamines (PEs). By combining high resolution mass spectrometry and nuclear magnetic resonance spectroscopy it was possible to deduce the structures of several intermediates indicating an oxidative cleavage of the Amadori product yielding glycoxidized PEs including advanced glycation end products, such as carboxyethyl- and carboxymethyl-ethanolamines. The pro-oxidative role of glycated PEs was demonstrated and further associated with several cellular responses including activation of NFκB signaling pathways. Label free proteomics indicated significant alterations in proteins regulating cellular metabolisms. Finally, the biophysical properties of PL membranes changed significantly upon PE glycation, such as melting temperature (Tm), membrane surface charge, and ion transport across the phospholipid bilayer.