Effect of lipid peroxidation on protein conformational stability

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https://doi.org/10.17952/37EPS.2024.P1296

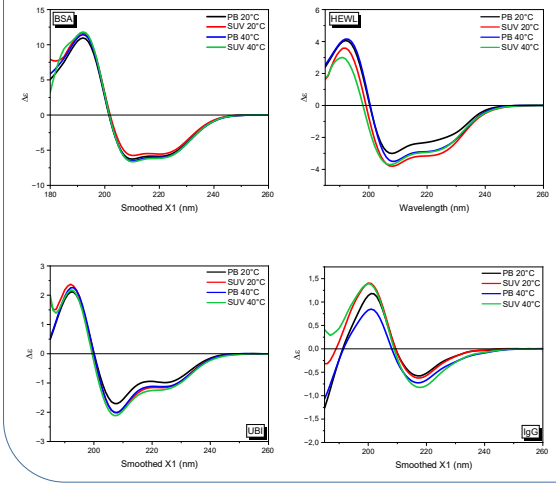
Introduction

Lipid oxidation can occur in vivo during oxidative stress and redox imbalance conditions, such as inflammation or environmental toxicity. This process generates reactive oxygen species (ROS), including the superoxide anion and hydroxyl radical within cells. Unsaturated fatty acids in tissues are particularly prone to oxidation by these ROS through non-enzymatic mechanisms. Lipid peroxidation, operating via a chain mechanism, produce a wide range of oxygenated small molecules, which significantly affects membrane organization and can modify proteins and DNA, leading to functional changes [1]. To study the effects of lipid peroxidation on the conformational stability of model proteins, BSA, HEWL, Ubiquitin, and rabbit IgG were solubilized in phosphate buffer and examined alone or in the presence of DMPG-DOPC (at a 1:1 molar ratio) small unilamellar vesicles (SUVs) at either 20°C or 40°C.

Synchrotron Radiation Circular Dichroism (SRCD) and the SRCD UV-denaturation assay

Oxidative conditions were created by collecting 30 consecutive repeated synchrotron radiation circular dichroism (SRCD) spectra at beamline B23 of the Diamond Light Source synchrotron (Didcot, UK).
The synchrotron radiation, besides increasing the S/N ratio and expanding the available spectral window, due to its high photon flux and brilliance is able to induce water photolysis and production of ROS, which can denature protein/peptide folding [2].
This method is used to quantify the UV photostability of proteins/peptides with and without ligands as a function of the environment [3].

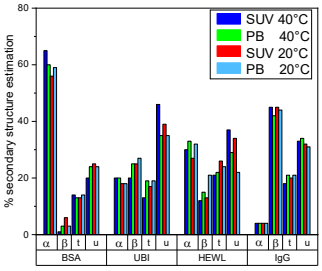
Effect of lipids and Temperature



Three mainly helical proteins were analysed in different environments, and taking the spectrum of proteins in phosphate buffer at 20°C as reference,

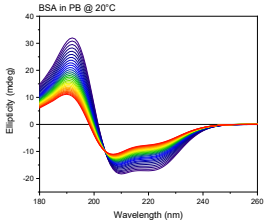
- BSA is the least influenced by the change of solvent polarity and temperature, as all spectra are almost superimposable
- UBI shows a slight increase in the intensity of both negative bands, comparable in all other environments
- HEWL shows increased intensity of the negative bands with temperature, and a similar effect with lipid presence, while the intensity of the positive band is decreased both with lipids and with temperature

IgG is characterized by a predominant β-sheet folding, with a positive band at ~205 nm and a negative one at ~220 nm.
The intensity of these bands is affected differently by heating or the presence of phospholipids.
In PB at 40°C the intensity of the positive band decreases, and the negative one increases. The latter is not affected by lipids at 20°C, while it is slightly increased in lipids at 40°C. On the contrary, the positive band increased in intensity at both temperatures studied when in presence of lipids.



The secondary structure content estimation performed using both CONTILL and CDSSTR algorithms implemented in the CDApps software [5] shows no significant variation in protein conformation related to heating and/or addition of phospholipids.

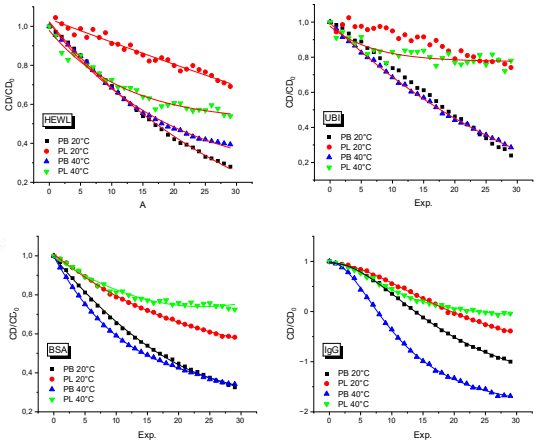
SRCD UV-denaturation



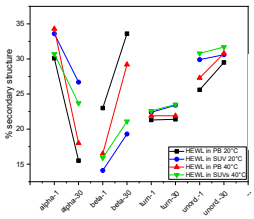
Protein UV-denaturation assays were performed in the presence or not of DMPG:DOPC SUVs.
30 consecutive SRCD scans were recorded at 20° or 40°C. The progressive decrease in the intensity and/or change in shape of the CD bands indicates a progressive loss of the native structure of proteins due to the action of reactive oxygen species generated by the photolysis of water molecules as previously demonstrated [3].

The change in the CD spectra is more evident in the buffer solution than in the presence of DMPG:DOPC SUVs. The increment of temperature (40°C) has a positive effect on the rate of denaturation in the presence of only the buffer. In the presence of SUVs a decrease in the rate of change of the SRCD was detected at the increasing of temperature.

Plotting the ellipticity values at λ_{max} vs the scan number:



Fitting the ellipticity values at λ_{max} vs the scan number with an exponential decay function (Origin ExpDec1: $y = y_0 + Ae^{-x/t}$), allows to determine both the decay rate ($k = 1/t$) and the half-life ($t½ = t * \ln 2$) from the time constant parameter (t). Linear (UBI in PB and PL 20°C) or sigmoidal (IgG) fitting do not allow to estimate k or t½.
Higher decay rate and lower half-life reveal a rapid process.



The estimation of secondary structure before and at the end of the UV-denaturation experiment shows that the loss of native conformation is mitigated in the presence of SUVs.

	k = 1/t				t½ = t * ln2			
	HEWL	UBI	BSA	IgG	HEWL	UBI	BSA	IgG
PB 20°C	3,08	n.d.	5,06	Sigmoidal fit	22,52	n.d.	13,70	Sigmoidal fit
PB 40°C	5,368	2,66	8,27		12,89	26,08	8,38	
SUVs 20°C	0,38	n.d.	4,53		n.d.	n.d.	15,31	
SUVs 40°C	8,33	14,60	11,30		8,32	4,74	6,16	

Conclusions

For the investigated proteins, CD spectra revealed that their conformation is not affected by temperature change or lipid presence.
The SRCD UV-denaturation assay showed that the presence of lipids seems to prevent the denaturation of proteins, which opens new questions on the effects of the synchrotron light on the lipids structure, stability and reactivity, which will be addressed by future studies.

References

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