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## **Succinimide formation in lysozyme by heating (80°C) in the dry state decreases protein stability but do not impact on secondary or tertiary structure.**

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Chemical modifications of proteins occurring naturally are of great importance to understand changes in polypeptide stability, loss of protein function and finally mechanisms of degradation. Among these reactions, deamidation of asparagines and isomerisation of aspartyl residues have been shown to occur *via* succinimide formation.

In this work, lysozyme was used to determine whether succinimide formation was possible in a polypeptide chain during heating in the dry state (80°C). This way, up to five cyclic imides appeared on the same protein after 7 day dry-heating. These succinimyl polypeptides were separated by cation exchange chromatography. The modified residues were identified by mass spectrometry Asp18, Asp48, Asp66, Asp101 and Asn103. The resulting molecules seemed to undergo no secondary and tertiary structure modifications as assessed by circular dichroism and intrinsic fluorescence experiments. This can be explained by the fact that all the identified modifications are located in loops. However, guanidinium hydrochloride induced unfolding curves showed that stability of the proteins decreased as a function of the number of modifications carried by the polypeptide chain. Moreover, binding of ANS was greatly enhanced suggesting an increase in surface hydrophobicity and/or a higher molecular flexibility.

We conclude that dry-heating is a good way to produce enriched succinimyl protein samples without important structural modifications. The resulting peptides exhibit lower stability and increased surface hydrophobicity. These results are important to understand the process of ageing of proteins and work is in progress to assess whether such modifications could modify fibril formation of lysozyme.

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