Characterization of photographic gelatin by 2D-electrophoresis

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Abstract

Age related changes were studied by 2D-PAGE. It shows that the gelatin molecules break down into smaller fragments with ageing. Also a broadening of the range of isoelectric points is seen.

Zusammenfassung

Alterungsbedingte Veränderungen wurden mit Hilfe der 2D-PAGE (Zweidimensionale Polyacrylamidgel- Elektrophorese) untersucht. Sie zeigt, dass die Gelatine- Moleküle bei der Alterung zu kleineren Fragmenten abgebaut werden. Ausserdem erweitert sich der Bereich der isoelektrischen Punkte.

Introduction

The deterioration of photographic gelatin can be studied by 2-dimensional polyacrylamide gel electrophoresis (2D-PAGE) where changes in the isoelectric point and the molecular weight of the molecules are revealed on a gel. The result is a 'map' of spots, where each spot represents a single peptide. The deterioration was studied by comparing maps of new and aged materials.

Materials

Gelatin has been used for photographic emulsions since 1871 and it still dominates modern photographic emulsion making. Most photographic gelatins are derived by alkaline hydrolysis of bone collagen (ossein) or cattle hides, followed by thermal extraction of the gelatin in water. The gelatin solution is then evaporated, cooled, gelled and dried. During the subsequent emulsion making process the gelatin is often hardened (e. g. with formaldehyde) to reduce the swelling and solubility of the emulsion. A hardener can also be added during processing after film exposure.

The collagen to gelatin transition results in disintegration of the triple helix structure into randomly coiled polypeptide chains, breaks in the backbone of the collagen molecule and cleavage of inter and intra molecular crosslinks. This produces a mixture of single (α), double (β) and triple (γ) stranded gelatins.

Samples have been taken from:

- Photographic gelatin IAG 5440 (Group Tessenderlo, PB Gelatins)
- Processed B&W film (AGFA APX25), unaged
- Processed B&W film (AGFA APX25), accelerated aged (60°C/70%RH/30days)
- Historical glass plate negatives, one in good condition from the1950's and one deteriorated i. e. stained and bleached from the 1920's

Sample preparation

New gelatin can be solubilized by swelling in water followed by heating to about 40°C. Aged gelatin or hardened gelatin is often more difficult to solubilize. The addition of urea can increase the solubilty of the sample. On the other hand, very degraded gelatin can be solubilized in cold water. In order to obtain consistency, all samples are allowed to swell 30 min. in a solution containing urea (also used for rehydration of IPG strips in isoelectric focusing) and heated 5-10 min. at 37°C. Amino acid analysis show that the solubility of the samples vary between 10-100%, and thus poses a serious problem in the 2D-PAGE procedure.

Equipment

For the first dimension, isoelectric focusing (IEF), the IPGphor Isoelecetric Focusing System, is used and for the second dimension, sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), the Multiphor II flatbed system is used (Amersham Pharmacia Biotech).

The isoelectric focusing uses immobilized pH gradient strips covering the range from pH 3-10 or 4-7. For the SDS-PAGE we used gradient gels 8-18%, covering a range from 6,500 to 300,000u.

Low molecular weight marker globular proteins were run, to estimate the molecular weight of the gelatin molecules. The separated proteins were visualized by silver staining.

Results and discussion

On the 2D map of unaged photographic gelatin, two α-bands at 95,000 and 96,000u, a β-band around 200,000u and a γ-band around 300,000 are present, together with a series of rows of spots around 50,000u and 30,000u. The molecular weight marker protein shows that 94,000u is somewhat be-

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low the α-bands. This discrepancy is due to the fact that globular proteins have a different mobility than fibrous proteins such as gelatin. The isoelectric point for the unaged gelatin is about pH 5 for the larger molecules. The rows of spots lie in the range from pH 5-7. When gelatin is aged there is a gradual loss of α-, β - and γ -bands. In the unagded processed black-and-white film the two α-bands are present, together with the spot pattern around 50,000u. With accelerated ageing the α -bands disappear, while the spot pattern remains intact, slightly intensified. In addition new spots appear in the aged pattern around 50,000u and pI 6-7. Also in around 20,000u a few new spots can be detected. The 2D maps of the historial glass plate negatives show the same spot pattern around 50,000u. On the map from the glass plate, which is in a rather good condition, also the reminiscence of the α-bands are seen.

Conclusion

2D-PAGE shows that α-, β- and γ-bands are present in new photographic gelatin. Furthermore, a spot pattern is seen around 50,000 and 30,000u. With ageing the high molecular weight proteins degrade into smaller fragments, enforcing the spot pattern around 50,000u, and adding spots to the lower molecular weight area. The pI range also broadens.

Biographies

Ulla B.Kejser graduated from the School of Conservation in 1993 with a MS degree in photographic conservation. Since then she has worked part time as lecturer at the School of Conservation and part time on research projects on the deterioration of photographic materials, leather, parchment and gelatin. In 1999 she was employed at the Royal Library of Denmark as a photographic conservator.

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