Location of the Free Thiol Group in Bovine β-Lactoglobulin A, B and C

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Abstract

Under non-reducing sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) conditions unheated samples of β-lactoglobulin (β-LG) A, B and C all run as a single band, the A variant having a slightly lower mobility than the B and C variants. Following heating of these samples to 110°C, two bands are seen in the monomer region of SDS-PAGE gels run under non-reducing conditions. As heat can induce disulphide exchange, the individual bands forming the doublet may represent species of the same molecular size but having different arrangements of the disulphide bonds. The band formed in the A variant as a result of heating appears to have the same mobility as the unheated B and C variants, while the band formed in the B and C variants as a result of heating appears to have the same mobility as the unheated A variant.

Under reducing conditions only a single band was seen in both heated and unheated samples, and the mobility of this band is the same in all three variants. This indicates that the difference in mobility between variants seen in non-reduced samples involves disulphide bonding. If the difference in the mobility of the two bands seen in heated samples is due to a difference in the position of a disulphide bond, and thus the free thiol, then it is possible that the position of the free thiol group in the A variant is different to that of the B and C variants even in unheated samples of this protein. A difference in the distribution of the thiol could explain observed differences in the reactivity of this group.

The purpose of this study was to determine whether the observed differences in the mobility of unheated samples of purified bovine β-LG A, B and C under non-reducing SDS-PAGE conditions is due to a difference in the location of the free thiol group within the primary sequence of these variants. This was achieved by reacting the β-LG variants with a radioactively labelled thiol-reactive reagent [1,4-^{14}C] N-ethylmaleimide (^{14}C-NEM), thereby attaching a radioactive marker to the free thiol group. Following labelling of the protein, carried out under conditions that did not induce band splitting,

the protein was hydrolysed and the resulting labelled peptide was purified and sequenced.

The free thiol group was found to be at residue 121 in β-LG A, B and C. Therefore differences in mobility during non-reduced SDS PAGE of β-LG A, B and C are not due to a difference in the location of the thiol group. However, results indicate that it is possible that, particularly in the B and C variants, there is a tendency for disulphide exchange to occur, even under relatively mild conditions.

In establishing the conditions under which band splitting did not occur, the effect of exposure to various conditions on the mobility of purified β-LG variants on native-PAGE and SDS-PAGE was studied. The mobilities of caprine β-LG and porcine β-LG were also studied in order to further characterise the factors within the primary sequence of β-LG that have an influence on band splitting.

With bovine β-LG A, B and C, band splitting was found to be both temperature- and pH-dependent. Protein concentration and the ionic strength of the buffer also appeared to effect band splitting. Heating also induced the formation of aggregated species, visible on both native and SDS-PAGE gels. The presence of aggregated material on SDS-PAGE gels indicates that disulphide bonding is involved in the formation of these species.

On native-PAGE, material that ran as a smear between the monomer band and dimer band was observed following heating. The protein present in this region may represent monomeric β-LG that has been sufficiently denatured for its mobility under native-PAGE to be retarded. Comparisons of the amount of material present in monomeric forms under native and non-reduced SDS-PAGE suggest that multiple monomeric species of β-LG are present in heated samples.

Storage at -18°C in SDS-PAGE sample buffer was also shown to induce changes in the mobility of bovine β-LG A, B and C, and of caprine β-LG, on SDS-PAGE. Storage under these conditions caused the aggregation of β-LG but did not induce band splitting. The banding pattern in the dimer region of the stored samples differed

between the variants, with the A variant showing a banding pattern that was markedly different to that of the B and C variants and the caprine protein, which showed similar patterns. The bovine β-LG B and C and caprine β-LG showed similar tendencies to form aggregates, and had a greater tendency to form these high molecular weight species than β-LG A. These differences may be due to a difference in the reactivity of the free thiol group under these conditions, influenced by the substitution at position 118.

Purified, unheated caprine β-LG ran as a single band in non-reduced SDS gels, and appeared to have the same mobility as the unheated bovine B and C variants under these conditions. Heating of caprine β-LG also induced the formation of a second band with a similar mobility to that of unheated β-LG A. Caprine β-LG has an Asp at position 64 (as found in bovine β-LG A) and an Ala at position 118 (as found in bovine β-LG B and C). The fact that in non reducing SDS-PAGE caprine β-LG runs as a band with a similar mobility to bovine β-LG B and C and a slightly higher mobility than bovine β-LG A suggests that the substitution at position 118 in the primary protein sequence may somehow be causing the mobility difference. Aggregated material was also seen in caprine β-LG following heating.

Unheated samples of porcine β-LG ran as two bands under non-reduced SDS-PAGE. Heating the porcine β-LG did not appear to induce any change in the appearance of the two bands, and there was no evidence of aggregation of this protein. Bovine β-LG A, B and C and caprine β-LG all contain a free cysteine residue in their protein sequence. Porcine β-LG does not contain a free Cysteine and thus the lack of heat-induced changes to the banding pattern in porcine β-LG when compared with the bovine variants and caprine β-LG is possibly due to the absence of this potentially reactive thiol group. The presence of a free thiol group appears to be required both to induce band splitting and for the formation of higher molecular weight aggregates following heating. Band splitting is thus probably a consequence of disulphide interchange reactions, the interchange reaction in β-LG A causing a second band to run in the position of β-LG Band C, and the interchange reaction in β-LG B and C causing a second band to run in the position of β-LG A.

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