The effect of processing method on the functional behaviour of globin protein

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Introduction

Conventionally haeme and globin have been separated with organic solvents, the acetone-method developed by Tybor et al. (1973) being the most famous. More recently Drepper et al. (1981) have described a method based on partial enzymatic hydrolysis of haemoglobin in the acid pH-range and Sato et al. (1981) a method based on carboxymethylcellulose (CMC)-column chromatography.

Functional characterization of acetone-treated globin prepared by the method of Tybor et al. (1973) has shown it to possess good water-binding capacity and emulsifying properties at pH 6 and lower but to lack the gelation property (Hermansson and Tornberg, 1976). Hayakawa et al. (1982) were the first to report that globin made by the CMC-column chromatography method forms a firm gel after being heated in the very narrow pH-range 5-6. Evidently the methods used to isolate globin has a significant effect on the functional properties of the final product.

The aim of the present study was to determine solubility, water-binding capacity and emulsifying and gelation properties for globin preparates made by the CMC-precipitation method (Autio et al., 1983, Autio, 1983). The effect of concentration and drying on the functional behaviour has been included in the characterization. The functional properties of preparates are compared with a native globin made by the method of Clegg et al. (1966).

Materials and methods

Preparation of globins: Bovine blood cell concentrate was obtained from a slaughterhouse in Helsinki, Finland, where the blood was collected under hygienic conditions and separated in an Alfa-Laval centrifugal separator. The separation of haemoglobin into haeme and globin fractions has been described by Autio et al. (1983) and Autio (1983). After separation the protein solution was frozen or freeze-dried, or concentrated by ultrafiltration (Paterson Candy International) to a dry matter content of 10 % and spray-dried. Globin was also prepared by the cold-acetone method as described by Clegg et al. (1966).

Thin-layer isoelectric focusing was performed on 0.5 mm polyacrylamide gels according to the method of Görg et al. (1980). About 25 % of LKB carrier ampholytes 7-9 and 8.0-9.5 were added to the gel. The pH-gradient was measured at 15 °C with a microglass electrode.

Solubility measurements were carried out as described by Lawhon and Cater (1971). The effect of pH and NaCl-concentration was studied. Determinations of solubility were carried out in duplicate.

Spontaneous uptake of water was measured with the Baumann apparatus as described by Hermansson (1979). The influence of pH and ionic strength was also studied. At least two duplicate experiments were made simultanously. The temperature of the sample was maintained at 15 °C by circulating thermostated water. Blanks were used to correct the evaporation.

Emulsifying activity (EA) and stability (ES) were determined according to the method of Yasumatsu et al. (1972). Two replications were prepared for every globin preparation at different pH-values and salt concentrations for both the ES and EA tests.

For the texture measurements of globin gels, 10 ml of globin solution was pipetted into tubes with an inner diameter of 25 mm. Total heating time was 30 min. Penetration measurements were made with the Instron Universal Testing Machine. A flat circular punch with a diameter of 5 mm penetrated the sample gel at a speed of 50 mm/min. Four replicates were made. The effect of protein concentration and pH on the penetration was studied.

Results and Discussion

An unfolding reaction of globin occurs when haeme is removed from the molecules, and as a result the isoelectric point of globin is higher than that of haemoglobin. In the focusing pattern two main bands, with isoelectric points of 7.7 and 8.0, were obtained for both the native and the CMC-treated globins. Further concentration of globin did not change the Ip's of globins. It seems that globin is quite stable against acid treatment and spray-drying.

The solubility curves of freeze-dried, spray-dried CMC-treated and native globins as a function of pH are presented in Fig. 2. Figure 3 illustrates the effect of NaCl-concentration. All globins showed a minimum solubility at pH 8.0. The native globin which had been prepared by the cold-acetone method was more soluble in the neutral pH than the CMC-treated globins. This difference is due to the higher salt concentration of globins made by the CMC-method. Even 1 % NaCl concentration drastically decreases the solubility of globin near the isoelectric point (Fig. 3). The same kind of solubility profile has been reported by Tybor et al. (1972) and Hermansson and Tornberg (1976) for globin made by the acetone-method (Tybor), with the exception that the solubilities were lower near the isoelectric points.

The water-binding capacities of globins as a function of pH and salt concentration are shown in Figs. 4 and 5. The spray-dried globin made by the CMC-method possesses greater water-binding capacity than the native and freeze-dried CMC-treated globins. This difference is probably due to the variations in porosity resulting from the different drying techniques. The water-binding capacities are greatest at acid pH-values although very good also at pH 7. One reason for the low water-binding capacity of freeze-dried globin may also be the high salt content in the dry powder. On the other hand the water absorption of spray-dried and native globin was higher in NaCl-solutions than in water at pH 5.5. Autio et al. (1984) have reported much lower water-binding capacities for porcine globine, especially in the presence of salts.

The emulsifying activity and stability are illustrated for native, freeze-dried and spray-dried globins in Figures 6a, 6b, 7a and 7b as a function of pH and salt concentration. The data reveal that with no added NaCl, both EA and ES are about equal for native and for spray-dried globin, whereas the ES is weaker for freeze-dried globin. The study of emulsifying properties at various pH-values showed that highest emulsifying activity and stability is around pH 4-5. At the same pH-value globin also forms a gel after being heated. In contrast to the results reported by Hermansson and Tornberg for acetone-treated globin (Tybor's method), the emulsifying properties of the globins studied here are quite good even at the isoelectric point. Salt decreased the emulsifying properties of native and spray-dried CMC-treated globin, having a much greater effect on the former.

Native and CMC-treated globins formed a firm gel at the concentration of 3 % at pH-value 5-6. The force necessary for penetration of globin gels was greatest for the CMC-treated globin that had not been dried. It formed a gel at 1.5 % concentration. At lower protein concentration the gelation took place at higher pH-value of 6-6.5. The relationship between force of penetration and protein concentration is illustrated in Fig. 8 and that between force of penetration and pH in Fig. 9. It has been reported that globin made by the method of Tybor et al. (1973) does not form a gel (Hermansson and Tornberg, 1976). The gelation property seems to be a good indicator of the denaturation of globin.

The very good water-binding capacity and the emulsifying and gelation properties of the present globin allowed it to be used especially in semisolid and solid meat products.

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Fig. 1 Electrofocusing pattern of native and CMC-treated, spray-dried bovine globins

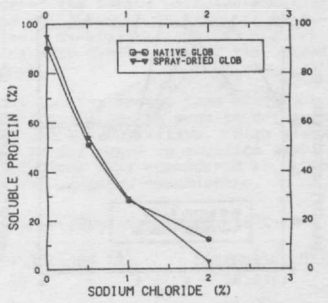

Fig. 3 The solubility of globins in water as a function of NaCl concentration.

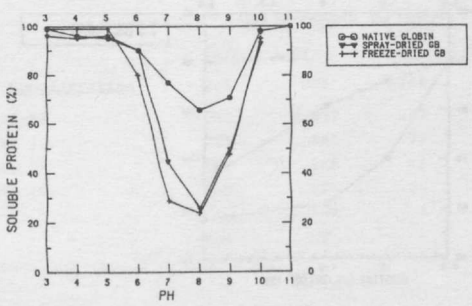

Fig. 2 The solubility of globins as a function of pH

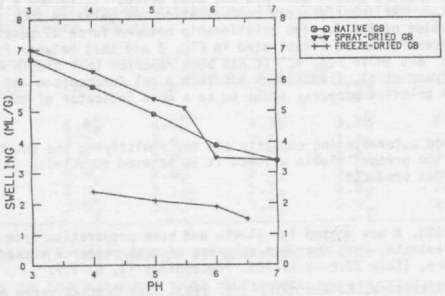

Fig. 4 The swelling of globins as a function of pH

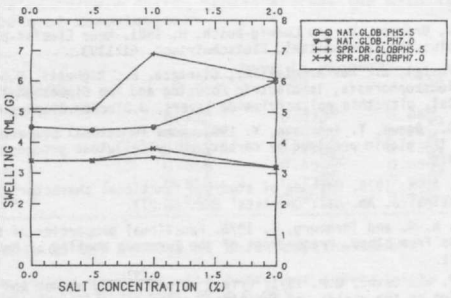

Fig. 5 The swelling of globins as a function of NaCl-concentration

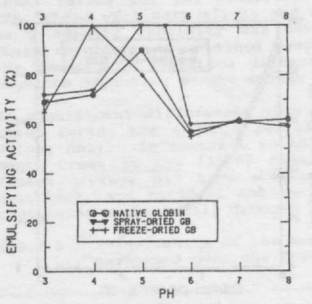

Fig. 6a The emulsifying activity of globins as a function of pH

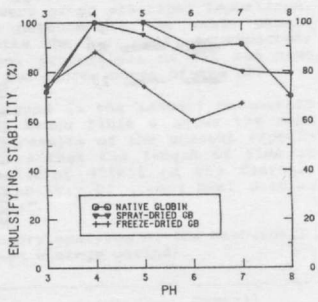

Fig. 6b The emulsifying stability of globins as a function of pH

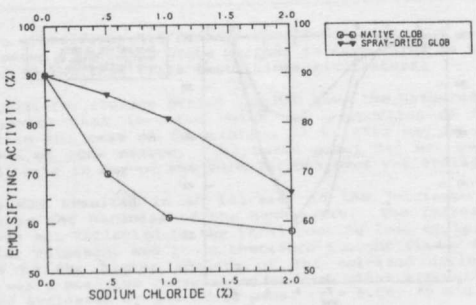

Fig. 7a The emulsifying activity of globin as a function of NaCl concentration

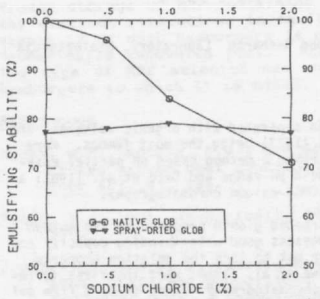

Fig. 7b The emulsifying stability of globins as a functions of NaCl-concentration

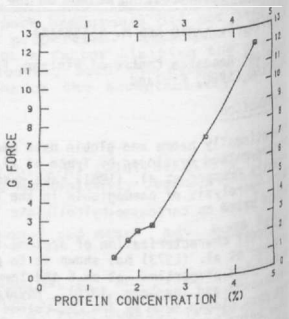

Fig. 8 Strength of globin gels (maximal force of penetration) as a function of protein concentration

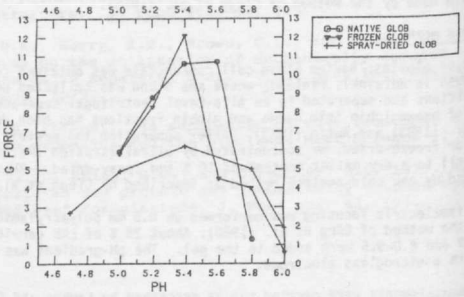

Fig. 9 Strength of globin gels as a function of pH.