INTERACTIONS BETWEEN K-CARRAGEENAN AND MYOGLOBIN

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SUMMARY

Visible spectroscopy was used to study the behaviour of metmyoglobin in dilute solution at pH 4.5 to 8 in the presence of k-carrageenan. At 25°C and pH values of 5.6 or less the polysaccharide induced complete denaturation of the protein and at higher temperatures promoted the thermal denaturation of metmyoglobin at all pH values studied.

The reactions were very dependent on the ionic strength and pH of the system and it was concluded that electrostatic forces are largely, but not entirely, responsible for the results observed. The significance of such protein - polysaccharide interactions in meat products is discussed.

INTRODUCTION

The behaviour of the k-carrageenan molecule with respect to pH, metal counter-ion type and concentration and with other polysaccharides has been extensively studied (Whistler 1973) and, although not completely understood, has been keenly exploited commercially. However, the interaction of k-carrageenan with proteins has received relatively little attention and even this has been confined to studies involving the highly specific interaction with k-casein (Snoeren et al. 1975) and the less specific interaction with gelatin (Lii 1978).

Very little work has so far been devoted to the study of the behaviour of carrageenan in the presence of other proteins associated with meat and meat products. A single study (Howell et al. 1984) has observed a pronounced effect of k-carrageenan inclusion on the gel strength of porcine blood plasma. This observation, together with studies from our laboratories (e.g. Ledward 1979) and by Tolstogusov and co-workers (e.g. Muchin et al. 1978), on the interaction of alginate and pectate polysaccharides with various meat derived proteins suggest that significant interactions may occur between k-carrageenan and some such proteins.

In addition, charged polysaccharides have been used to bind restructured meats (Means and Schmidt 1986) and thus some understanding of their mode of action is desirable. The present paper describes some studies on the interaction of k-carrageenan and the well characterised, water soluble protein, metmyoglobin.

MATERIALS & METHODS

Sodium k-carrageenan was obtained from Ceca SA (Lot SE1927/B2) and atomic absorption spectrophotometric analysis indicated it contained 6.03% sodium, 0.12% potassium, 0.31% calcium and 0.11% magnesium. The chloride ion content was less than 0.03% and thus the polysaccharide was used without further purification. The sodium salt was chosen as it is cold water soluble and sodium is known to interact less with the polysaccharide chain than the other common counter ions, potassium and calcium (Morris and Chilvers 1983). Horse heart metmyoglobin (essentially salt free) was obtained from Sigma Chemical Co. (Lot 52F 0460).

SPECTROSCOPY

Absorption spectra - the salt region was determined on 0.01% w/v solutions while 0.1% w/v solutions were used to study the spectra at higher wavelengths, 460-660 nm. pH was adjusted with HCl or NaOH. Such addition led to volume increases of less than 1%. Spectra were recorded with a Perkin-Elmer Model 576 3T double beam spectrophotometer equipped with a thermostatically controlled sample cell. The path length was 1 cm and the scan speed either 100 nm/min (340-460 nm) or 200 nm/min (460-660 nm).

RESULTS

1. Effect of pH

In the pH range 5.0 to 7.0 the spectra of metmyoglobin was independent of pH; at pH 7.9 there was some slight decrease (≈5%) in the Soret peak at 409 nm due to dissociation of the water molecule at the 6th co-ordination position of the haematin, pk 8.93.

However, in the presence of an equal concentration of sodium k-carrageenan a marked pH dependence was noted (Fig. 1), typical of urea denatured myoglobin (Ledward 1971). It is clear from Fig. 1 that the Soret maximum does not undergo a gradual shift in wavelength

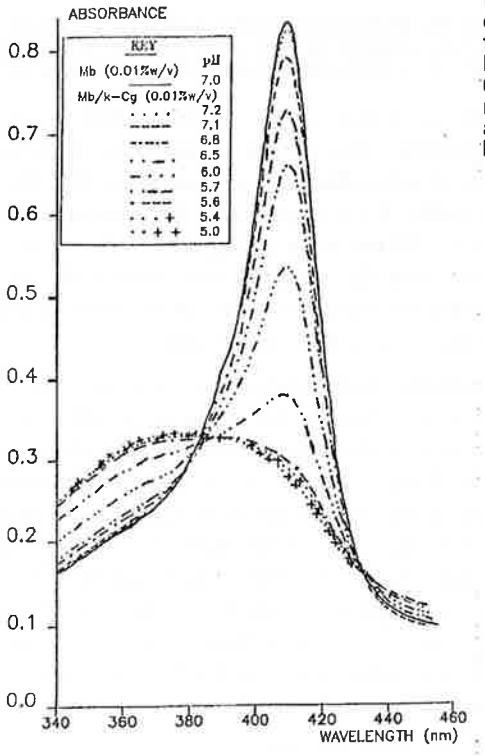

Figure 1: The effect of pH on the interaction between 0.01% metmyoglobin and 0.01% k-carrageenan.

with decreasing pH, rather the pattern is typical of a mixture of two components of differing concentration. This interpretation is supported by the existence of the isobestic points at 383 nm and 432 nm.

These observations are consistent with a one-step denaturation of myoglobin, which proceeds without stable intermediates. If so, then the apparent equilibrium constant will be proportional to Cn (Schechter and Epstein 1968), i.e.

$$\log k = \text{constant} + n\log C$$

where C is the denaturant concentration. Also the concentration of metmyoglobin at a given pH will be proportional to the absorbance at 409 nm or the ratios of the absorbance at 409 nm to that at 383 nm or of that at 409 nm to that at 432 nm or inversely proportional to the absorbance of 374 nm (peak maximum for the "denatured species"). Thus apparent equilibrium constants can be calculated and the logarithm of these are plotted against the log of the hydrogen ion concentration (-pH) in Fig. 2.

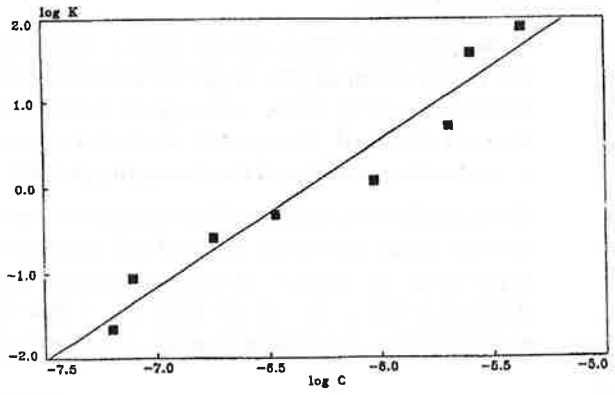

Figure 2: The relationship between hydrogen ion concentration, [C], and the apparent equilibrium constant, K, for the reaction between 0.01% metmyoglobin and 0.01% k-carrageenan.

The linear nature of this relationship supports the proposed one-step model, in which the k-carrageenan induces denaturation of metmyoglobin with the release of haematin. At these pH's both haematin and denatured globin would normally precipitate and thus it must be assumed that the charged polysaccharide, as well as perturbing the native structure of the protein, effectively maintains the products in solution.

Although the model of the denaturation process fits the experimental data, it has been used differently to its application to denaturation using urea (Schechter and Epstein 1968) since the concentration of hydrogen ions present in the system has been used as the denaturant concentration. This implies, incorrectly, that the hydrogen ions are directly responsible for the denaturation process. However, the use of pH was a convenient device, equivalent to calculating the charge distribution on the macromolecules, and it does illustrate the importance of electrostatic forces in the interaction.

2. Effect of ionic strength

Salt, either KCl or NaCl, markedly inhibited the interaction, thus at pH 5.05 in the presence of 0.5M salt there was little or no evidence of any interaction. The effect of ionic strength on the absorbance at 409 nm on solution of 0.01% metmyoglobin and 0.01% carrageenan at pH 5.05 and 25C is shown in Fig. 3. It is seen that NaCl

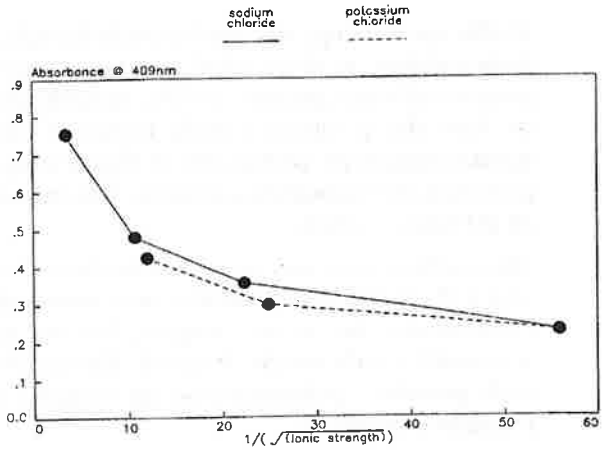

Figure 3: The effect of ionic strength upon the interaction.

is slightly more effective than KCl in inhibiting the interaction and this may be because the excess sodium ions suppress the dissociation of the sodium k-carrageenan and thus reduce the charge on the polysaccharide and the effectiveness of its interaction with the protein.

3. Effect of temperature

On its own myoglobin showed little spectral change until denaturation occurred (at about 80°C at pH7) and the solution became turbid. However, in the presence of k-carrageenan marked changes were apparent at lower temperatures. At pH 7.04 the spectra exhibit a reduction in maximum absorbance with increasing temperature (Fig. 4) and the wavelength of maximum absorbance decreased steadily from 409 to 403 nm. Unlike the interaction at pH 5 and 25°C (Fig. 1) no peak appeared around 374 nm and when solutions of the two macromolecules at pH values of 5 and below were heated the spectra typical of "free haematin", with a peak at 374 nm, was lost (Fig. 5).

No precipitation occurred in any of the carrageenan containing systems. It would thus appear that although the nature of the products may differ, decreasing pH and increasing temperature both increase the strength of the k-carrageenan - myoglobin interaction, inducing denaturation of the protein and production of soluble haematin and non-haematin containing moieties.

DISCUSSION

As with pectate protein and alginate protein systems, it would appear the electrostatic forces dominate the interaction between these polysaccharides and the protein. Thus, the effect is more marked at lower pH

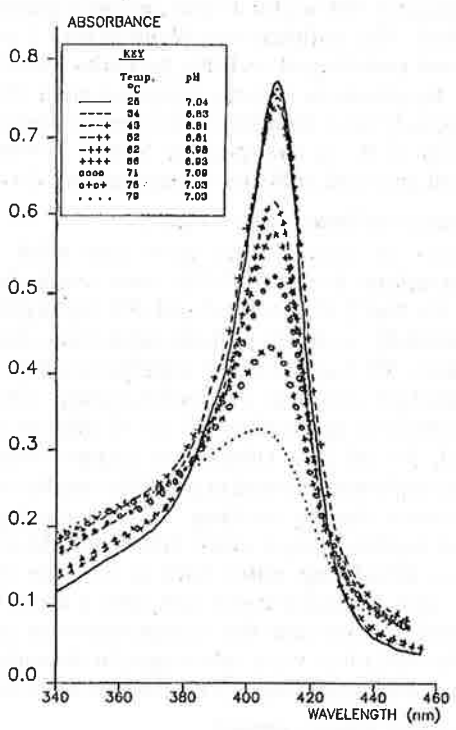

Figure 4: The effect of temperature on the interaction between 0.01% metmyoglobin and 0.01% k-carrageenan at pH 7.04.

values (where the protein will carry a higher positive charge). However, total charge is not the sole criterion since its distribution on the protein will also be important (Wills 1986) as will the flexibility of the polysaccharide chain (Ledward 1979). The implication of this work to the commercial use of charged polysaccharides in meat products is rather tenuous since significant interactions only occur at low ionic strength (i.e. 0.01M), a condition not normally found in meat products. However, it is established that at high ionic strength charged macromolecules may interact, though not electrostatically, to modify the expected behaviour of the individual species and Castelain et al. (1986) have proposed a mechanism for bovine serum albumin - carboxymethyl cellulose systems involving protein-protein aggregation induced by volume exclusion phenomena.

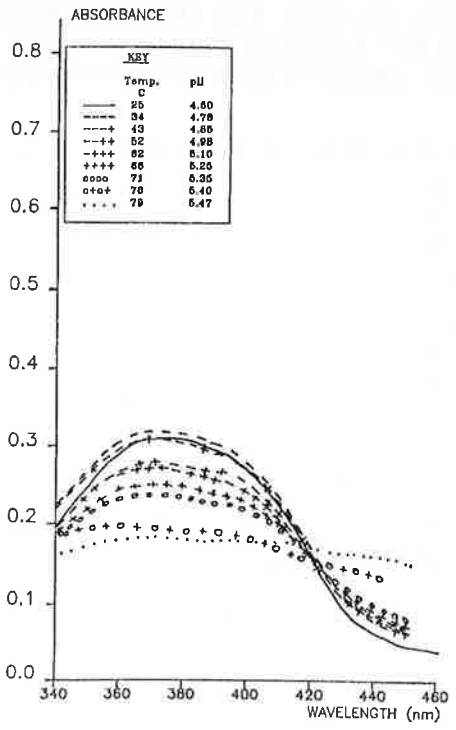

Figure 5: The effect of temperature on the interaction between 0.01% metmyoglobin and 0.01% k-carrageenan at pH 4.60.

In addition, Howell and Laurie (1984) found that at high ionic strength k-carrageenan alginate and pectate significantly decreased the strength of blood plasma gels. The effect was less at lower ionic strength and thus cannot be explained in terms of intermolecular, electrostatic interactions between the macromolecules. If no intermolecular interactions take place, then the systems may be considered as a "co-gel" with separate protein rich and polysaccharide rich domains. In such a "co-gel" the macro gel properties, such as breakstrength, will be determined by the interactions of the two gel networks rather than by specific protein-polysaccharide interactions (Clark and Lee-Puffrel 1984). Such aspects of protein-polysaccharide mixed systems need to be studied if maximum benefit is to be obtained from the use of these macromolecules.

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