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INTERACTION OF LACTOSE WITH INDIVIDUAL MILK PROTEINS AND THERMODYNAMICAL PARAMETERS. I. K-CASEIN

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INTRODUCTION

Heating is by far the most important of all steps in milk processing. All dairy products are subjected to some sort of heat treatement.

It is therefore not surprising that much effort has been done in order to elucidate the physical and chemical efects of heat in milk, and particulary to study the carbohidrates - proteins interaction, thought to be of primary importance in the browing reaction. This subjet has been excellently reviewed by Patton.[9]

Work by several groups (1.4.5.10) have proved the interaction of lactose with milk protein as a whole, when skim milk is heated from 60 to 98 C. and higher. The contribution of any of the individual milk proteins to the binding of lactose is however unknown and no attempt has been made to calculate the thermodynamic parameters of this interaction.

This is the first of a series of papers aiming to obtain information about the number and nature of the binding sites of the interacting protein, the relative ability of the different milk proteins and specially the individual components of bovine casein to interact with lactose and, the thermodynamic parameters of this interaction.

MATERIALS AND METHODS

K-casein was prepared from isoelectric casein (Merck) by the procedure of Tripathi and Gehrke.[11] Its purity was verified by polyacrylamide and starch gel electrophoresis. The molecular weight of casein was taken as 20,000 and correction were made to allow for its water content. Lactose, NaH_2PO_4, $NaHPO_4$ and anthrone were supplied by Merek. Mixed K-casein and lactose solutions in phosphate buffer were heated at temperatures from 60 to 98 C for 20 min. Casein was held constant ($1{,}5 \times 10^{-4}$ M) lactose ranged from 2.9×10^{-3} to 2.9×10^{-2} M. Ionic strength 0.2 and pH 6,9 were held constant. After heating, the experimental procedure followed closely that already described by PATEL[8]. Samples were dialyzed against the buffer used for heating. Dialysis cells were of the type described by PATEL and Foss.[7] Cellulose membranes (Fisher Scientific Company) were held at 90 C in destilled water for one hour and washed with the same solvent to remove any water soluble contaminant. Dialysis equilibrium was attained after 10 hours at 20 C with constant agitation. After the equilibrium has been reached lactose was determined at both sides the membrane by the anthrone reaction. Extintions were read with a Bausch and Lomb Spectronic-20, at 620 m μ. Readings from the inner side of the membrane were corrected for the carbohydrate content of K-casein. Lack of breakage and subsequent diffusion of part of carbohydrate moiety of K-casein was verified at every temperature.

RESULTS AND DISCUSSION

From the law of mass action, assuming no interaction among bound ions, we can easily derive:

$$r = \sum_i \frac{k_i\, c}{1 + k_i\, c} \qquad (i = 1,2,\ldots n) \qquad (I)$$

where n = number of binding sites per protein molecule, ki = intrinsic association constant, r = moles of lactose per mol of protein, and c = free lactose concentration.

If the intrinsic association constants were the same for every polymer site, this equation could be reduced to:

$$r/c = nk - r\,k \qquad (II)$$

where, n = number of available binding sites per molecule of protein.

Plotting r/c against r a straight line is obtained from which *n* and k can be culated, since the slope = —k and the intercept with the r/c axis = = nk. Furthermore the intercept with the r axis = n, since it can be deduced from (I).

$$\lim_{c \to o} r/c = \sum_i k < i$$

All other thermodynamic parameters can be calculated from k by the following equations:

a) $$\triangle F^o = -R\,T \ln k$$

where $\triangle F^o$ = free energy change, R = the gas constant and T= absolute temperature.

b) The van't Hoff differential equation:

$$\frac{d \ln k}{d\ T} = \frac{\triangle H^o}{R\ T^2}$$

written as follows:

$$\log \frac{k_2}{k_1} \quad \frac{\triangle H^o}{2.303\ R} \quad \left(\frac{1}{T_2} - \frac{1}{T_1}\right)$$

where $\triangle H^o$ = free enthalpy change of the sistem.

and c) $$\triangle F^o = \triangle H^o - T\ \triangle S^o$$

where $\triangle S^o$ = enthropy change.

Figure 1 shows the plot of the experimental results after heating at 70, 80, and 98 C. At 60 C the interaction was barely detectable. The values obtained for the intrinsic association constants and the thermodynamic parameters of the lactose —K-casein interaction are in table I.

Our work shows for the first time the contribution of an individual protein to the heat —induced lactose-protein interaction in milk. It is generally assumed that this interaction finally results in browning our heat treatments did not produce in any case detectable browning, that showing that the interaction mesured is previous or independent of the browning reaction. Furthermore the binding extent diminishes with increasing temperatures between 70 and 98 C (k decreases along this temperatures range from 28 l. mol^{-1} to 8.6 l. mol^{-1}). This result does not agree with what is known about the relationship between heating temperature and browning of molk, and the reaction of other casein fractions.[6]

n values are not discrete numbers; this may be due, either to experimental error in dialysis or lack of molecular homogeneity of the protein in its

binding behavior, as it has been shown by KARUSH[3] for bovine serum albumin.

Enthalpy change varies with temperature but we have not sufficient data to define the enthalpy change-temperature relationship. For our enthalpy change calculations, we have assumed that $\triangle H^o$ is not temperature dependant from 70 to 80 C, but when temperature is increased from 80 to 98 C k values decrease less than could be expected for a constant $\triangle H^o$ value.

The index of the last column of the table gives the amount of water diffusing across the membrane by osmosis, and proves that the number of K-casein particles decreases when temperature increases from 70 to 98 C.

The temperature induced change in the aggregation state, negatively affects binding ability of the protein.

The high negative value of the free enthalpy change ($-\triangle H^o = 12.1$ Kcal. mol^{-1}) is consistent with a nonionic interaction; the predominant contribution to it must be due to van der Waals forces.

From the negative enthalpy change it may be deduced that these forces are energetically stabilized by dipole-dipole interactions which is a comon phenomenon in the interaction between proteins and neutral molecules.

We have notestabilished the nature of groups in the protein molecule to which lactose is bound, but HODGE and RIST[2] have postulated that interaction between sugars and protein occurs predominatly on the epsilon amino group of lysine, which may be the case here.

The enthropy change of the system follows a normal thermodynamics as it was to be expected that lactose-protein complex formation decreases the enthropy of the system.

RESUMEN

Se ha realizado el estudio termodinámico de las interacciones entre K-caseína y lactosa. K-caseína, electroforéticamente pura (5×10^{-5} M) se calentó a pH = 6,9 y fuerza iónica 0,2 (tampón fosfato) en presencia de lactosa ($2,9 \times 10^{-3}$ M — $2,9 \times 10^{-2}$ M) durante 20 minutos en el intervalo de temperaturas de 60º a 98º C. Las muestras fueron dializadas y determinada la cantidad de lactosa enlazada. El análisis matemático mediante la ecuación de Scatchard, suponiendo iguales las constantes de asociación intrínseca para todos los lugares de enlace en la molécula de proteína, permitió el cálculo de los cambios de energía libre $\triangle F^o$, entalpia $\triangle H^o$ y entropia $\triangle S^o$. A 60º C el enlace no es detectable. Cuando la temperatura se incrementa de 70 a 98º C, la asociación lactosa proteína decrece así como la extensión del enlace, demostrando que se trata de un proceso exotérmico. Los parámetros termodinámicos del proceso sugieren que la mayor contribución al mecanismo de la interacción es debida a fuerzas de van der Waals. El alto valor del cambio de entalpia (—12,1 Kcal. mol^{-1}) indica que las fuerzas implicadas están fuertemente estabilizadas por interacción dipolo-dipolo. El cambio de entropia (—30 u.e.) es el que normalmente cabría esperarse de un proceso de esta naturaleza.

RESUME

On a efectué une étude thermodinamique sur les interactions entre la K-caséine et la lactose. La K-caséine, électrophorétiquement pure, (5×10^{-5} M) fut chauffée à pH 6,9 et force ionique 0,2 (buffer de phosphate) en présence de lactose ($2,9 \times 10^{-3}$ M — $2,9 \times 10^{-2}$ M) pendant 20 minutes à un intervalle de température de 60 à 98º C. Les échantillons furent dialysés et l'on calcula la quantité de lactose unie.

L'analyse mathématique, au moyen de l'équation de Scatchard, en supposant les constantes d'association intrinsèque égals pour tous les points d'union dans la molécule de protéine, permit de calcular les changements d'énergie libre $\triangle F^o$, entalpie $\triangle H^o$ et entropie $\triangle S^o$. A 60º C l'union n'est pas détectable. Quand la température augmente de 70º C à 98º C, l'association lactose protéine diminue ainsi que l'extension de l'union, ce qui démontrenqu'il s'agit d'un procédé exothermique.

Les paramètres thermodinamiques du procédé suggérent que la plus grande contribution au méchanisme de l'interaction est due à des forces van der Waals. La valeur élevée du changement d'entalpie (—12,1 Kcal. mol^{-1}) indique que les forces impliquées sont fortement stabilisées par interaction dipôle-dipôle. Le changement d'entropie (—30 u.e.) est celui que l'on pourrait habituellement attendre d'un procédé de cette nature.

SUMMARY

A thermodynamic study of K-casein - lactose interaction was made. Electrophoretically pure K-casein (5×10^{-5} M) was heated at pH 6,9 and ionic strength = 0,2 (phosphate buffer) in presence of lactose

($2{,}9 \times 10^{-3}$ M — $2{,}9 \times 10^{-}$ M) for 20 min at 60 to 98 C. Heated samples were dialyzed and bound lactose determined. Mathematical analysis by the Scatchard equation, assuming equal intrinsic association constants for all binding sites of the protein molecule. allowed calculation of free energy, enthalpy and enthropy changes ($\triangle F^o$, $\triangle H^o$ and $\triangle S^o$). At 60C, binding is barely detectable. From 70 to 98 C the protein-Lactose association constant decreases as well as the extent of the binding, proving that this is an exothermic process. The thermodynamic parameters of the binding suggests that the main contribution to the interaction mechanism is due to van der Waals forces. From the highly negative value of the enthalpy change (—12.1 Kcal mol^{-1}) it is deduced that the implied forces are strongly stabilised by dipole-dipole interaction. The change of enthropy (— $\triangle S^o$ = 30 enthropy units) was negative as expected.

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TABLE I

Association constant and thermodynamic parameters of the binding of lactose by K-casein in phosphate buffer; pH = 6.9; μ = 0.2.

Temp. C	N.° sites n	n k 10^2	binding constant k 1/mol	enthalpy change $\triangle H^o$ kcal/mol	free energy change $\triangle F^o$ kcal/mol	enthropy change $\triangle S^o$ e. u.	osmosis index (a)
70	9.9	2.7	28.03		— 2.26	— 28.6	0.6
80	8.7	1.5	17.3	— 12.08	— 1.99	— 28.6	
98	13.3	0.96	8.6		— 1.41	— 28.9	0.4

(a) Weight increment of the dialysis tube without protein minus weight increment of the dialysis tube with protein.

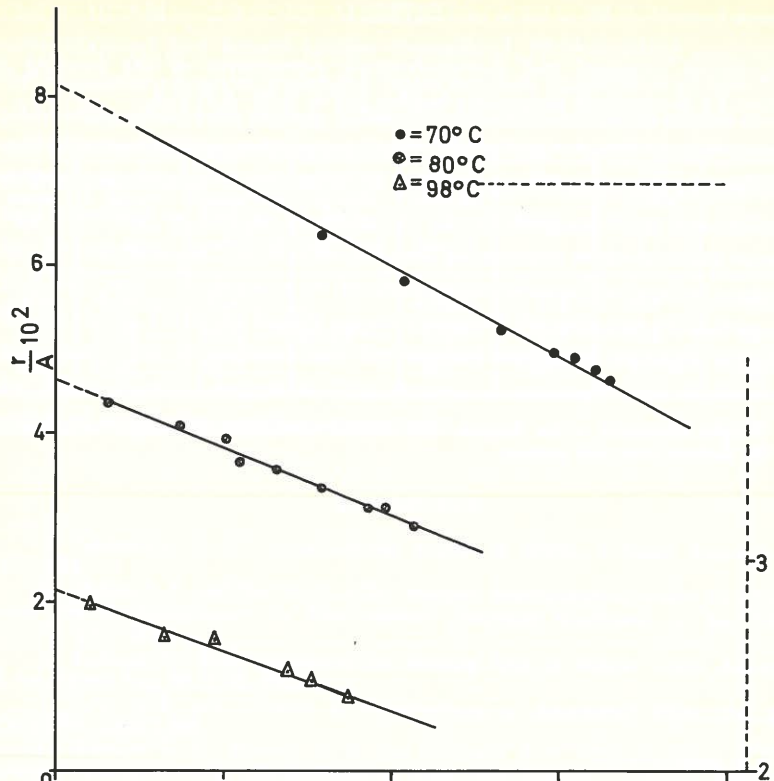

Figure 1

Fig. 1.—Scatchard plot of lactose and kappa casein binding after 20 min heating at 70 and 98 C in phosphate buffer. Lactose concentration 2.9×10^{-3} M — 2.9×10^{-2} M. Protein concentration 5×10^{-5} M ionic strength 0.2; pH 6.9.