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STUDIES ON WATER RETENTION AND WATER RELEASE FROM SOME PROTEIN SYSTEMS

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The hydrogen-bond properties (WBI index), water retention and water release from the protein-water systems gluten--water, soya protein-water and casein-water, have been investigated using differential scanning calorimetry in the temperature range 223-423 K. The proteins were characterized by their isoelectric point, contents of carboxyl groups and sulfur-containing groups, and readiness of undergo chloromethylation. It was concluded that the marked difference in water-release behaviour is chiefly explained by conformational differences and charge effects.

The steric structures and general physical properties of protein-water systems are largely determined by the water molecules present and the hydrogen-bond network formed by them. Owing to the theoretical and practical importance of these properties for protein-water systems in vivo and in vitro, a great amount of work has been done to characterize the nature of the bonding of water in proteins [1]. Despite these investigations, our knowledge regarding the above questions is still very unsatisfactory.

The methods used to investigate the hydrogen-bond properties of protein-water systems may be divided into four groups: thermodynamic methods, kinetic methods, spectroscopic methods and diffraction methods.

Owing to the strong influence of hydrogen-bonds on thermodynamic properties, especially convincing results have been ob-

tained using calorimetry in wide temperature intervals. With the advent of differential scanning calorimetry /DSC/, the measurements have become rapid and easy to perform [2].

On the basis of experimental evidence, the water present in protein-water systems is generally divided into three classes:

/a/ Constitutional water is strongly bound to the amino acids of the protein structure and shows anomalous physical behavior.

/b/ Interfacial water contains hydrogen-bonds which are directed either to the interior of the macromolecule or to its outer parts. Interfacial water is intermediate in behavior between constitutional water and

/c/ bulk water, which seems to be quite weakly influenced by the protein system.

It is often very difficult to distinguish between the various types of water bonding in protein systems. Therefore, for practical purposes a simple division into two types, weakly and strongly protein-bonded water may be preferred [3].

In a study concerning the bonding properties of gluten, soya protein and milk casein in composites, it was found that exact and comparable data concerning water retention and water release from these proteins were lacking. We have therefore carried out an investigation of this subject using scanning calorimetry. Owing to experimental difficulties the approach was purely thermodynamic, but a kinetic treatment of the phenomena is under development [4].

EXPERIMENTAL

Materials

Wheat gluten /vital wheat gluten manufactured by Raisio Factories, Finland/, soya protein /Purina Protein Europe/ and milk casein /Bulgarian origin/ of technical quality were used after purification with water and/or modification by carboxymethylation. The proteins and their derivatives were characterized by their isoelectric point, content of nitrogen, content of carboxyl groups, content of sulfur-containing groups and infrared spectra. Cystine was used as reference compound.

The modification of the proteins was performed by carbomethylation, using monochloroacetic acid as reagent. A suspension of 150 g protein in 600 ml distilled water, was made basic /pH 11/ with 7.5 g sodium hydroxide and digested at 313 K for 4 h with 7.5 g monochloroacetic acid. The reaction was stopped by lowering the pH to 8 and the reaction product was precipitated at pH 5. The precipitate was purified by repeated treatment with distilled water and centrifugation.

Methods

The carboxyl contents of the unmodified and modified proteins were determined with the calcium acetate method [5]. The isoelectric point was determined by weighing the amount of dry matter precipitated as a function of pH and taking the derivative of the precipitate vs. pH curve [6].

The thermodynamic measurements on the protein-water systems were performed with a Perkin-Elmer DSC-2 scanning calorimeter in the temperature range 223-423 K. The proteins were carefully mixed with distilled water and the mixtures were stored for 24 h in sealed ampoules in an ice-box before use.

The calorimetric measurements were made in pans with perforated lids. The rate of heating was 5 degree min^{-1} and the sensitivity of the instrument was adjusted to 20 $mJ.s^{-1}$. The enthalpy of vaporization of water, ΔH, was calculated from the integrated area of the deflection from the base line. All data were referred to the vaporization of pure water under similar experimental conditions.

The amount of strongly-bound water was calculated as a WBI index [7], which indicates in per cent proportion of the heat of vaporization due to the strongly hydrogen-bonded molecules:

$$WBI = 100 \left(1 - w/W \right) \qquad (1)$$

where WBI indicates the percentage of bound water, w is the area corresponding to the pure water, and W is the area for the strongly-bound water in the protein-water system. The total amount of water should be equal for both cases.

RESULTS AND DISCUSSION

The analytical data on the compounds and systems are given in Table 1 and the corresponding thermodynamic data, including the WBI index, are given in Table 2.

From Table 1 it is evident that the proteins studied show a marked difference in properties with regard to their carboxylic group content and especially to the readiness of the amino acids to undergo further carboxylation. Within the limits of experimental error, the carboxyl content of gluten is much lower than those of soya protein and casein. On the other hand, after carboxylation the end-products show roughly equal carboxyl contents (cf.[8]).

A comparison of the infrared spectra of the proteins before and after carboxymethylation indicates that in all modified proteins marked depolymerization also takes place during the reaction. The degradation is likewise evident from the polarographic data for sulfur-containing groups in proteins. Initially, in gluten and casein the contents of sulfur-containing groups are about equal, but after the carboxymethylation the reduction in the amount of the groups is much more marked in gluten than in casein. The differences are clearly explained by the monomer structure in the proteins, which is well known from sequence analyses [9,10].

According to Ewart [11] the three-dimensional character of the gluten macromolecule may be caused by the presence of disulfide bonds, -S-S-, which are easily detected by polarography /cf. Table 1/. Rheological data do not support the hypothesis of Ewart, but rather the view that the three-dimensional macromolecular structure is caused by non-covalent bonds and principally hydrogen-bonds [12]. The small change in the pH of the isoelectric point after modification also seems to support the latter view. The fibrillar structure proposed for gluten as compared to the miscellar structure of casein seems to favor water retention.

A comparison of the thermodynamic data in Table 2 for the three protein-water systems reveals several important facts. The WBI index of Karmas and DiMarco [7] shows a general trend

Table 1

Analytical data on proteins and their modifications

Modification	Water,[1] %	N, %	Isoelectric[2] point, pH		COOH groups meq.g^{-1}		Polarographic data[3] $-E_{1/2}$, sh cm.10^{-7}			
			before	after	before	after	before	after	before	after
gluten	6.7	13.5	5.3	5.2	0.279	1.441	0.82	0.78	1.8	0.3
soya protein	5.1	13.0	5.5	5.3	0.790	1.292	-	-	-	-
casein	4.4	13.8	5.2	5.4	0.733	1.535	0.64	1.28	1.7	0.8
cystine[4]		11.9					0.77	-	1.4	-
cysteine[5]		11.6					1.20[6]			

[1] Air-dry sample.

[2] Determined by precipitation as a function of pH.

[3] sh relative intensity for the polarographic wave, $E_{1/2}$ half-wave potential, concentration 10 g.l^{-1}, pH 14, temperature 298 K. The proteins were only partly dissolved. The measurements were therefore performed in suspensions.

[4] 26.7% sulfur.

[5] 26.5% sulfur.

[6] pH 10, 12.6 mole% cystine.

Table 2

Enthalpy of vaporization, $\Delta\underline{H}$, and relative water binding intensity index, WBI, as functions of water content and temperature of protein-water systems

	$\Delta\underline{H}$, $J.kg^{-1}$			WBI,%		
Protein	Gluten	Soya	Casein	Gluten	Soya	Casein
H_2O,%	T = 323-342 K			H_2O,%	T = 223-423 K	
10	3.80	4.00	4.20	0	100	100	100
20	2.80	4.00	4.20	20	100	100	95
30	1.75	0.80	1.35	40	80	95	90
40	1.65	0.95	1.45	60	60	70	35
50	1.75	1.15	1.35	80	20	47	45

to decrease with increasing water content. This implies that the weak interactions increase more rapidly than the total amount of water in the system, which may be explained by a partial uncoiling of the macromolecule. This decrease is especially marked in the casein-water system for water contents greater than 50%. The difference in behaviour between casein and the other two proteins is not simply related to differences in isoelectric point, acidic group content or solubility. It seems likely that chiefly conformational changes and charge effects may explain the variances observed. This hypothesis is supported by kinetic experiments which are still in progress.

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ZUSAMMENFASSUNG - Wasserstoffbrückenbindugseigenschaften (WBI-Index), Wasserretention und Wasserabgabe von Protein-Wasser-Systemen - Gluten-Wasser, Sojaprotein-Wasser und Casein-Wasser - wurden im Temperaturbereich von 233-423 K mit einem Scanning-Kalorimeter untersucht. Die Proteine wurden durch ihren isoelektrischen Punkt, den Gehalt an Carbonylgruppen und Schwefel enthaltenden Gruppen und durch ihre Reaktivität in der Chlormethylierung charakterisiert. Es wurde gefolgert, dass der ausgesprochene Unterschied im Wasserabgabeverhalten in erster Linie auf strukturelle Unterschiede und Ladungseffekte zurückzuführen ist.

Резюме - Методом дифференциальной сканирующей калориметрии были исследованы в области температур 223-423 К свойства водородной связи, удерживание и выделение воды в системах протеин - - вода, глютен - вода, соевый протеин - вода и казеин - вода. Протеины были охарактеризованы их изоэлектрической точкой, содержанием карбоксильных и серосодержащих групп, а также способностью к реакции хлорметилирования. Сделано заключение, что заметное различие в характере удерживания воды обусловлено конформационными различиями протеинов и влиянием заряда.