STRUCTURAL AND FUNCTIONAL MODIFICATION OF RAPESEED ALBUMIN BY ACETYLATION

Klaus Dieter Schwenke, Kim Yung Hak**, Barbara Raab, Wilma Pähtz, Angelika Krajewski, Dietrich Zirwer*, Erika Lange and Gerhard Mieth

Zentralinstitut für Ernährung, Potsdam-Rehbrücke, Zentralinstitut für Molekularbiologie*, Berlin-Buch, Akademie der Wissenschaften der DDR und Institut für Ernährung der medizinischen Akademie der KDVR**, Pjöngjang

1. Introduction

The low molecular mass basic albumin fraction is a main storage protein besides the 12 S globulin in rapeseed (BHATTY et al., 1969, LÖNNERDAL et al., 1972, SCHWENKE et al., 1973). It comprises a group of homologous proteins with a molecular mass of about 14 000 g/mol, built up of a small and a large disulphide-bridged polypeptide chain, each. Owing to its high percentage this protein fraction contributes considerably to the functional properties of the total of rapeseed proteins.
During the last years the chemical modification, especially the acylation of reactive groups, has become an interesting tool for improving the functional properties of food proteins. Rapeseed protein isolates have been modified by acetylation and succinylation (THOMPSON et al., 1984). Recently, the influence of succinylation on the structure and functional properties of the 12 S globulin (SCHWENKE et al., 1986, NITECKA et al., 1986) and the low molecular basic albumin fraction (NITECKA et al., 1986) from rapeseed has been investigated. The present paper concerns the influence of acetylation on chemical and physico-chemical properties of the albumin fraction.

2. Results and Discussion

The modification was performed by addition of acetic anhydride to the protein solution in phosphate buffer (pH 8.0). Thereby the acetylation of ε-amino groups of lysine takes place quickly in the presence of a small excess of the reagent (Figure 1).

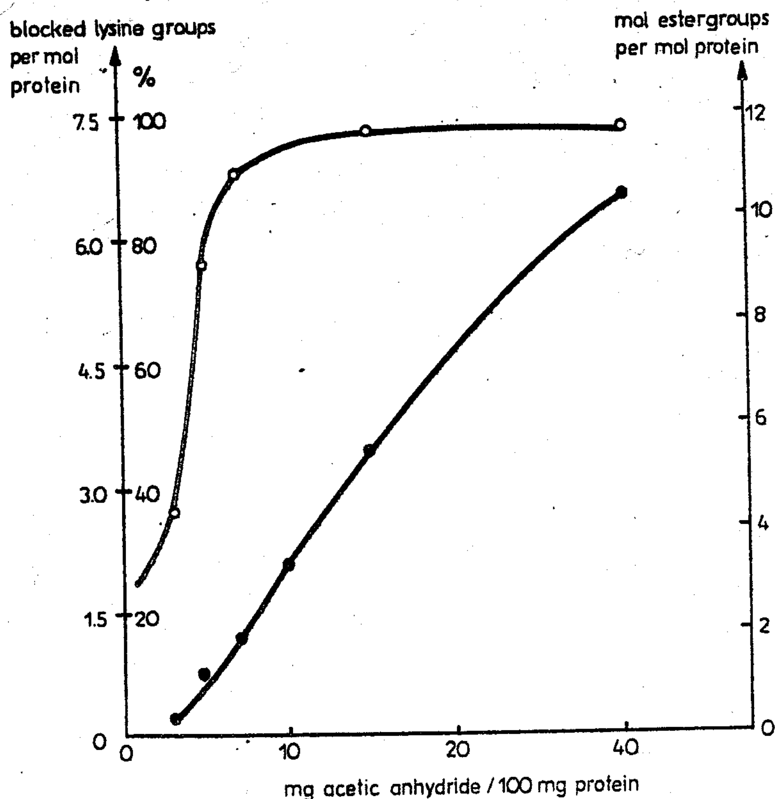

Figure 1. Dependence of lysine blocking and hydroxyamino acid esterification on the amount of acetic anhydride
(O) lysine blocking, (●) hydroxyamino acid esterification

Simultaneously an esterification of hydroxyamino acids takes place. The N-acetylation proceeds, however, remarkably faster than the O-acetylation. That means that at an exhaustive (98 %) acetylation of the ε-amino groups of lysine, only 50 % of hydroxyl groups are esterified. The simultaneous but unequal acetylation of amino acid hydroxyl groups demands the determination of all blocked groups for the characterization of acetylated food proteins. With regard to the reproducibility of protein processing and properties, one has to take into consideration the content of ester groups, too.

The secondary structure, characterized by a high α-helix content of about 40 % (SCHWENKE et al., 1975), does not change after acetylation. On the other hand, some changes in the near ultraviolet CD spectrum and a quenching effect in the fluorescence spectrum may point to some alterations in the tertiary structure. These changes proceed farther after the exhaustive N-acetylation until all hydroxyl groups are blocked. They point to a certain flexibility of the albumin. The latter is limited owing to the stabilization by disulphide bridges. Therefore, no shift of the fluorescence emission maximum is observed in exhaustively acetylated samples.

The viscosity dependence on the degree of acetylation resembles that of the succinylated albumin (NITECKA et al., 1986 b): The high intrinsic viscosity of the native protein drops after blocking of amino groups owing to the reduction of electrostatic repulsion in the molecule. It reaches a minimum at moderate degrees of acetylation (Table 1). This may reflect rather the change of molecular shape due to the reduction of intermolecular electrostatic repulsion after acetylation of polar groups than conformational changes.

A continuous increase of surface hydrophobicity with increasing number of acetyl groups in the protein has been observed by means of the ANS-probe fluorescence technique.

Contrary to that, the high interfacial activity of the native protein on the water-oil interface does not change by the modification (Table 1).

The distribution of polar and nonpolar groups on the molecular surface and a sufficiently high flexibility of polypeptide segments may contribute to the excellent foaming properties of the native protein (NITECKA et al., 1986 b). The influence of "hydrophobing" the protein by acetylation on foam capacity and stability is to be studied yet.

The unchanged interfacial activity of the acetylated protein may point to a negligible effect of acetylation on the emulsifying properties of the albumin.

The blocking of about 80 % of the protein amino groups results in a total inhibition of the heat-induced aggregation. Therefore, the acetylation is similar to the succinylation (NITECKA et al., 1986 b) of considerable benefit for the regulation of heat-aggregation of the albumin.

Table 1

Intrinsic viscosity $[\eta]$, surface hydrophobicity[1)] difference between the native and modified proteins ΔS_0 and interfacial tension π [2)] of the albumin depending on the degree of acetylation of amino groups

degree of modification of amino groups (%)	$[\eta]$ (dl/g)	ΔS_0	π (mN/m)
0	0.159	0	26.3
36	0.038	80	26.2
53	0.046		
83		150	26.2
93		170	26.2
98 (50 % esterif.)		260	
98 (90 % esterif.)	0.055	370	25.2

1) determined by means of ANS (anilino-naphtalene sulfonic acid) fluorescence probe technique

2) determined by the trop-volume method

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