COMPOSITION OF THE FATTY ACID FRACTION OF ZEINS WITH VARIOUS DEGREES OF PURIFICATION

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It has been shown that maize zeins are associated with lipids mainly of saturated nature. The process of quantitative repression of prolamines in maize grain under the action of the mutant gene opaque-2 is accompanied by a change in the associative properties with substances of lipid nature. A covalent nature of the zein polypeptide–lipid (fatty acid) bond is suggested.

Zeins – the alcohol-soluble reserve proteins of maize grain – are synthesized in the membranes of protein bodies and contain small amounts (0.1-1%) of carbohydrates [1-3] and lipids [2-5]. The sites of glycosylation of the zein polypeptides have been studied. It has been shown that some subgroups of zein are glycoproteins. The glycosylation of zeins presumably has a fundamental role in the process of post-translational modification of the proteins [6]. Little is known about the lipid components of zeins. In the IEF-components of the zeins lipoproteins have been detected [2] in the pH range of ∿3, amounting to 3-5% of the total population of the zein. They contain a covalently bound carotenoid which is, apparently, a component of the membrane surrounding the protein bodies. It has been shown that the fatty acids present in the lipoprotein complex of zeins change the physicochemical properties of this group of proteins and, in particular, their capacity for being precipitated from aqueous alcoholic solutions [4, 5]. In a study of lipids–zeins interaction the methods of staining [6] and extraction by polar solvents [4, 5] have been used but, as will be shown below, this is not adequate.

If the presence of a covalent peptide–acylglycerol(fatty acid) bond is assumed, then it is necessary to use methods cleaving this bond with breakdown into the components and the subsequent analysis of the lipid components obtained. We have therefore used the method of analyzing the fatty acid (FA) composition of bound lipids [7] which includes the hydrolysis of the sample, extraction of the FA fraction, and its analysis in the form of methyl esters by the GLC method. The amount of FAs was determined after the successive stages of isolation and purification of the zein: I) modified alcoholic extraction from a flour of the grain; II) reprecipitation with acetone; III) (main fraction) after gel filtration on Sephadex LH-20; IV) boiling in acetone with activated carbon. The initial and mutant forms of maize A 204 o2/o2 that we described in [8] were used.

In a modified extract of the zeins (Table 1, column 1) about 13% of FAs was detected, their qualitative composition being characteristic for the total lipids of maize grain [9]. With respect to their quantitative ratio they differed from the latter, since in the total grain lipids unsaturated FAs predominated, while in the zeins their ratio to the saturated FAs had decreased considerably. After reprecipitation of the protein with acetone, the amount of FAs in the zein preparations fell by a factor of 16-17.

In the fractions of zeins after column chromatography (Table 1, column III), the amount of FAs had scarcely changed in comparison with that after reprecipitation with acetone (Table 1, column 2). Boiling with activated carbon gave a degree of purification 5-7 times greater than in the preceding operation. The splitting out of the unsaturated FAs took place more intensively than that of the saturated FAs in the process of purifying the zein from stages I to IV. If we compare the degree of decrease in the level of FAs in the purification process from stage I to stage IV, for the saturated FAs it amounts to a factor of 22-26, and for unsaturated ones a factor of 123-267.

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TABLE 1. Amounts of Fatty Acids in Maize Zeins at Various Stages of Purification (g per 100 g of sample)

Fatty acid	Stage of purification							
	I		II		III		IV	
	+ +	o2/o2	+/+	o2/o2	+/+	o2/o2	+/+	o2/o2
$C_{14:0}$	0,0645	0,052	0,030	0,011	0,030	0,014	0,011	0,01
$C_{16:0}$	2,210	2,403	0,363	0,193	0,294	0,313	0,091	0,080
$C_{18:1}$	3,655	3,624	0,184	0,212	0,143	0,252	0,058	0,012
$C_{18:2}$	6,980	6,813	0,238	0,320	0,214	0,452	0,028	0,027
$\Sigma_{saturated}$	2,2745	2,455	0,393	0,204	0,324	0,327	0,102	0,092
$\Sigma_{unsaturated}$	10,635	10,437	0,412	0,532	0,357	0,704	0,086	0,039
Σ_{FAS}	12,910	12,892	0,805	0,736	0,681	1,031	0,188	0,131

The presence of saturated FAs in zeins reduces the value of the method of detecting lipid components by staining, since the latter is the result of an interaction of a dye with the double bonds of the FA molecules. Various results (Table 1, columns III and IV) on the amounts of FAs in zeins of the initial maize and that converted onto a mutant basis indicates that the process of repressing the prolamines under the action of the mutant genes is not only quantitative but is accompanied by a change in the associative properties with nonprotein substances.

An additional treatment of the zein after stage (IV) from a mixture of ether and chloroform which, in the opinion of Abe [5], completely eliminates lipids was performed. However, hydrolysis of the preparations before and after this purification of a zein liberated identical amounts of FAs. The treatment of the zeins with Folch's mixture [7] after stage (IV) of purification likewise did not lead to a decrease in the amount of FAs.

The insignificant differences in the amounts of FAs contained in different purified preparations of the zein, permit the assumption of a covalent nature of the FA (lipid)—zein bond.

EXPERIMENTAL

Maize grain was ground in a ÉM-3a mill. The flour was defatted three times with petroleum ether and was dried in the air. The proteins were extracted with a fivefold volume of 70% ethanol in centrifuge tubes with constant stirring at room temperature for 1 h followed by centrifuging at 3000g for 15 min. The solvent was evaporated off in a rotary apparatus (I). The dry residue was dissolved in the minimum volume of 70% ethanol.

The protein was precipitated by the addition of acetone cooled to −20°C (ratio of protein solution to acetone 1:9 by volume). The contents of the test-tubes were centrifuged at 3000g for 15 min. The precipitate was dried in the air until the smell of acetone had disappeared (II). A 100- to 150-mg sample of the zein was dissolved in 20 ml of 70% ethanol and the solution was deposited on a 2.6 × 60 cm column of Sephadex LH-20. The protein was eluted with 70% ethanol. The eluate was collected with the aid of a fraction collector and it was analyzed from its absorption in the ultraviolet and at 280 nm in a spectrophotometer. The first chromatographic fraction, the largest one, was isolated (III). Fraction (III) was treated with activated carbon, centrifuged at 13,000g for 15 min, and dried in vacuum (IV).

The hydrolysis of the zeins with alcoholic alkali was carried out as described in [7], and analysis of the fatty acids as in [8]. Table 1 gives the mean values of three repetitions.

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ESR SPECTRUM OF THE OXIDIZED VANADYLPORPHYRIN COMPLEX OF KARAZHANBASS PETROLEUM

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As a result of the use of various oxidants, mono- and bications of the natural vanadylporphyrins of Karazhanbass petroleum have been obtained, and their ESR spectra have been studied.

In [1], the electron spin resonance (ESR) spectra of vanadyletioporphyrin (VOEP) and vanadyltetraphenylporphyrin (VOTPP) and their various oxidized forms obtained under the action of Lewis acids and bromine are given.

In order to study the natural vanadylporphyrins (VOPs) present in the petroleum oils of the Caspian oil-bearing regions we have considered the ESR spectra of the complex isolated from Karazhanbass petroleum and the products of its oxidation. The vanadylporphyrin complexes were isolated by the extraction-chromatographic method and were identified from their characteristic absorption bands [2] on a Specord UV-Vis spectrophotometer at room temperature. The purity of the vanadylporphyrins isolated was determined from the absorption band at 408 nm (Soret band) and also on the basis of the absence of background absorption in the 400-600 nm region. The degree of purity of the samples obtained ranged between 80 and 95%. The complexes isolated consisted of a mixture of homologs of the vanadyl derivatives of deoxophylloerythroetioporphyrin (DPEP) and etioporphyrin (EP) [3]. The oxidants used were $SbCl_5$, $TiCl_4$, and Br_2.

The ESR spectra were taken in CH_2Cl_2 solution at 77 and 300 K on a Varian E-12 ESR spectrometer. Figure 1 shows the isotropic spectra at 300 K and Fig. 2 the anisotropic spectra at 77 K of vanadylporphyrin and its oxidized forms obtained under the action of various oxidants. An extract containing the complex VOP was obtained by the extraction-chromatographic method.

Table 1 gives the measured and calculated spectral parameters. The action of oxidants led to a decrease in the isotropic HFS constants (a_V) and in the anisotropic HFS constants A and B, and also to a change in the ratio of $g_\parallel$ and $g_\perp$ (in the initial complex, $g_\parallel < g_\perp$, and in the oxidized complexes $g_\parallel > g_\perp$). The change in the ratio of $g_\parallel$ and $g_\perp$ is reflected in the nature of the broadening of the HFS line in the isotropic spectra [1]. In the initial complex, VOP, the high-field components are broadened, while in the oxidized complex it is the low-field components. The values of $g_\perp$ and B for the oxidized forms were determined from the relations $g_{iso} = 1/3(g_\parallel + 2g_\perp)$ and $a = 1/3(A + 2B)$. A comparison of the results obtained with those given in [1] shows that the action of $SbCl_5$ and $TiCl_4$ apparently leads to the formation of bicationic complexes.

The action of bromine, as a weaker oxidant, probably leads to the formation of a monocation. As can be seen from Fig. 1, d, the spectrum consists of the superposition of the

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