X-Ray Structural Analysis of Talassimine. The space group and the parameters were established by the photo method and were refined on a Syntex $P2_1$ four-circle automatic diffractometer (CuK_α, graphite, monochromator, $\theta/2\theta$ scanning, $2\theta \leq 122.6°$): a = 9.885(3), b = 12.635(3), c = 15.219(3) Å, d_{calc} = 1.256 g/cm³; space group $P2_12_12_1$; Z = 4. The calculations were performed with 1640 reflections. The structure was interpreted by the direct method using the SHELXS-86 program [6] and was refined by means of the SHELX-76 program [7] in the anisotropic approximation. The positions of the hydrogen atoms calculated theoretically were included in the calculation. The final values of the divergence factors were R = 0.063 and R_w = 0.067. All the calculations were performed on a PC of the IBM PC/AT type. The coordinates of the nonhydrogen atoms of the molecule are given in Table 2.

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OLIGOMERS OF HUMAN SERUM ALBUMIN MODIFIED BY GLUTARALDEHYDE

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When human serum albumin is modified with glutaraldehyde, in addition to the trivial modification of the free amino groups of the monomer, the formation of dimers, trimers, and tetramers of albumin takes place through glutaraldehyde cross-linkages.

Interest in the modification of proteins by glutaraldehyde (GA) is due to the possibility of creating insoluble derivatives of enzymes with modified activity [1-3]. In addition, human serum albumin (HSA) modified by glutaraldehyde has receptors in the blood serum of patients suffering with type B viral hepatitis, and GA-HSA itself is considered as a mediator in the adsorption of the virus of hepatitis B on the plasmatic membrane of the hepatocyte [4, 5]. The chemical aspects of the modification of proteins by glutaraldehyde have been considered previously [6]. The present investigation was devoted to the oligomeric characterization of GA-HSA.

The elution profile of a solution of GA-HSA given in Fig. 1 shows the presence of three distinct bands. An analogous pattern in the fractionation of GA-HSA has been obtained previously [7, 8]. We selected the fractions corresponding to these bands (Fig. 1) and then analyzed them with the aid of electrophoresis in 7.5% polyacrylamide gel in the presence of 0.1% sodium dodecyl sulfate (Fig. 2). On electrophoresis of the fractions, four bands were revealed, corresponding to four forms of GA-HSA. Not one of the fractions was homogeneous. However, fraction I contained predominantly high-molecular-mass forms, fraction II predominantly a medium-molecular-mass form, and fraction III predominantly a low-molecular-mass form. The quantitative ratios between the forms of GA-HSA are given below (the amounts

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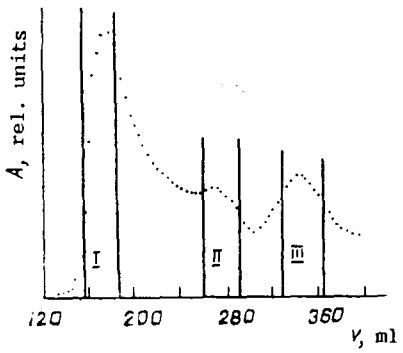

Fig. 1

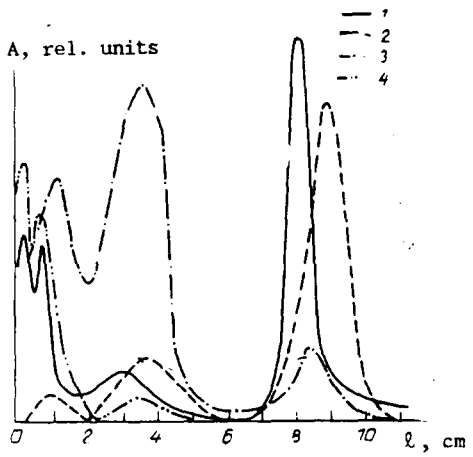

Fig. 2

Fig. 1. Elution profile in the gel filtration on Sephadex G-200 (0.1 M phosphate buffer, pH 7.4, 0.9% NaCl) of a solution of human serum albumin modified with glutaraldehyde. The Roman numerals show the fractions taken for analysis.

Fig. 2. Densitograms of plates of polyacrylamide gel (length 110 mm) after electrophoresis in 0.1% sodium dodecyl sulfate: 1) intact bovine serum albumin; 2) fraction III; 3) fraction II; 4) fraction I of human serum albumin modified with glutaraldehyde (Fig. 1).

of the oligomers were determined as the fraction of the area under the densitogram corresponding to each form):

Mol. mass kilodaltons	Oligomers	Fraction I	Fraction II	Fraction III
67	Monomer	—	10,6	74,0
134	Dimer	9,3	58,8	20,8
201	Trimer	48,4	30,6	5,2
268	Tetramer	42,3	—	—

When as fractions I, II, and III the volumes of eluate corresponding to the optical density maxima were taken, they were electrophoretically homogeneous (the predominant forms amounting to not less than 95%).

The heterogeneity of the fractions proved to be a convenient factor permitting the use of the individual forms as internal standards for determining the molecular masses, under the obvious condition that the molecular masses of the individual forms must be multiples of the molecular mass of monomeric HSA. The results presented in Fig. 3 show that the value of the decimal logarithm of the molecular mass evaluated as monomer, dimer, trimer, and tetramer of the albumin depends linearly on the R_f value in the electrophoresis of the albumins within the limits of experimental error. Therefore the correpsonding bands on electrophoresis may be regarded as mono-, di-, tri-, and tetra- GA-HSA, since otherwise the above-mentioned relationship would not be linear [9].

We may note that the corresponding bands of the GA-HSA fractions had somewhat greater R_f values than the bands of the intact serum albumin (this was shown most clearly for the band with the highest R_f value). Since the shift of the bands cannot be the consequence of differences in molecular mass, it must be assumed that the macromolecules in the GA-HSA fractions have more negative total electric charges than the charges in the corresponding fractions of the intact albumin. It has been shown previously [6] that glutaraldehyde interacts with a protein mainly at the free ε-amino groups of lysine and arginine, and also at the phenol group of tyrosine, the imidazole of histidine, and the sulfhydryl group of cysteine. The fall in the charge of the HSA after its modification by glutaraldehyde corresponds completely to the blocking of the free amino groups of the protein. The two active aldehyde groups of glutaraldehyde can react with the amino groups of two different HSA monomers, which leads to their cross-linkage and to the formation of oligomers. The

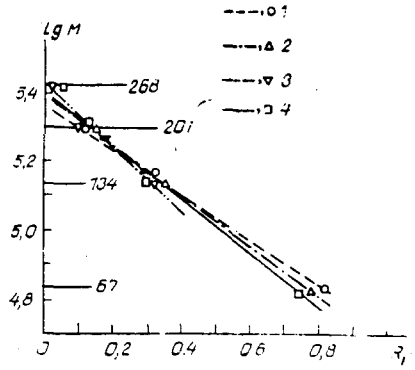

Fig. 3. Dependence of the decimal logarithms of the molecular masses (M) on the R_f values of the protein bands on the electrophoresis of fraction III (1), fraction (II) (2), and fraction (I) (3) of glutaraldehyde-modified human serum albumin and of intact bovine serum albumin (4) (see Fig. 2). The figures show the values of the molecular masses (in kilodaltons) of the monomer, dimer, trimer, and tetramer of the serum albumin.

possibility of intermolecular cross-linkages has been demonstrated by the formation of aggregates of ovalbumin with serum albumin [6].

EXPERIMENTAL

Commercial preparations of human serum albumin and glutaraldehyde from Reanal (Hungary), of bovine serum albumin from Sigma (USA), and of a set of reagents for electrophoresis in polyacrylamide gel from Serva (FRG) were used. Gel filtration was conducted in a 1.5 × 65 cm column filled with Sephadex G-200 gel. A standard set of equipment for liquid chromatography from LKB (Sweden) was used. The rate of elution was 22.5 ml/h, and the optical density at 277 nm was recorded. Vertical electrophoresis in plane polyacrylamide gel (dimensions of the gel 110 × 110 × 1 mm) in the presence of 0.1% sodium dodecyl sulfate (Serva, FRG) was carried out by Laemmli's method [10] with the aid of an AVGÉ-1 apparatus (Tallinn). Densitograms of the gel plates after staining with Coomassie Blue R were measured on an OE-503 scanning densitometer (LMM, Hungary). Molecular masses were determined by the method of Weber and Osborn [9]. Modification was achieved by adding to 3.6 ml of a solution of human serum albumin (20 mg/ml, 0.1 M phosphate buffer, pH 6.8) 0.4 ml of 2.5% glutaraldehyde in drops with constant stirring over 3-5 min followed by incubation at room temperature for 2 h [11]. After incubation, the solution was dialyzed against 0.1 M phsophate buffer, pH 7.4, in 0.9% NaCl and was deposited on a column.

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