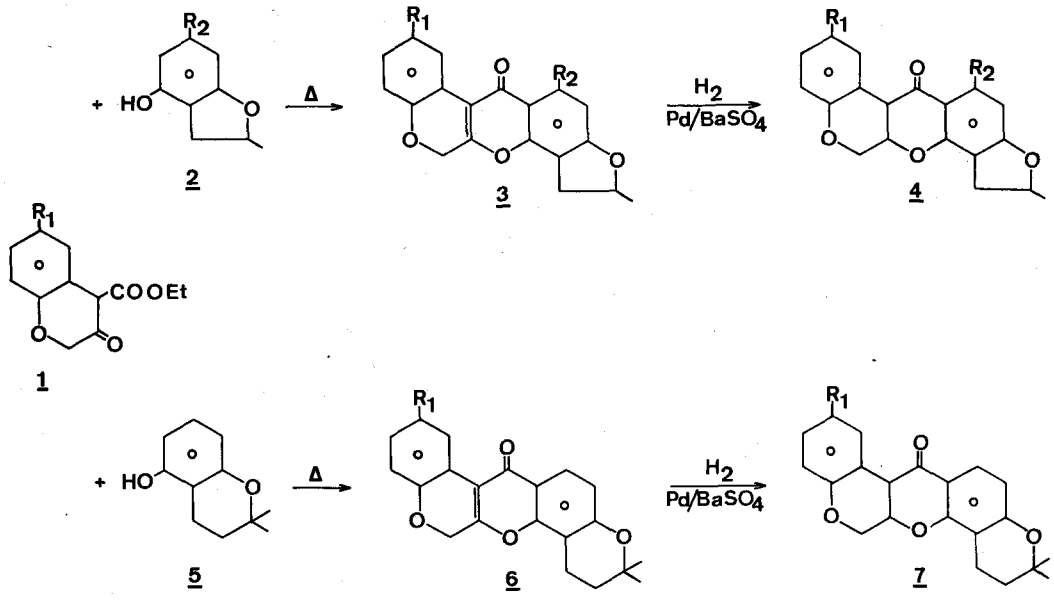
The IR-spectra of dehydrorotenoids displayed the typical pattern of a γ-pyron ring at 1650–1570 cm^{-1} (CO and C=C)[4]. The structure of **3** and **6** were confirmed by NMR and MS.

Hydrogenation of the dehydrorotenoids **3** (R_1=R_2=Me) and **6** (R_1=H) in a Parr apparatus with $Pd/BaSO_4$ in methanol at 4 atm. gave the rotenoids **4** (R_1=R_2=Me) and **7** (R_1=H). The rotenoid **4** (R_1=R_2=Me) was identical with a sample prepared by the MIYANO procedure[5], which consisted of the sodium borohydride reduction of the dehydrorotenoid **3** (R_1=R_2=Me) and successive Oppenauer oxidation.



Compound			Yield (%)	M.p.	IR (KBr) cm^{-1}
	R_1	R_2			
3	H	H	40	>300°	1635, 1605, 1560
3	Me	H	33	243°	1642, 1610
3	Me	Me	49	227°	1645, 1620, 1590
6	H	—	37	219°	1635, 1600, 1580, 1570
4	Me	Me	90	153°	1670, 1590
7	H	—	79	181°	1670, 1600

The IR of the rotenoids **4** and **7** now exhibited the carbonyl function at 1670 cm^{-1}, while the stereochemistry and the conformation was established by NMR confirming the *cis*-B/C fusion alloted to natural rotenoids[6].

Zusammenfassung. Eine einfache Synthese von Rotenoiden durch Kondensation von 4-Ethoxycarbonyl-3-chromanonen mit Phenolen und katalytischer Hydrierung der entstehenden Dehydrorotenoide wird beschrieben.

R. VERHÉ and N. SCHAMP

State University of Ghent,
Faculty of Agricultural Sciences,
Laboratory of Organic Chemistry,
Coupure 533, B-9000 Ghent (Belgium),
26 February 1975.

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Ultrastructural and Physico-Chemical Properties of a Polymeric Macromolecular Serum Protein

Much information has accumulated during the last decade concerning the fine architecture of many plasma proteins. Certain plasmaproteins, e.g. immunoglobulins and isoenzymes, can occur in polymeric form. We report here on the ultrastructure of a polymeric macromolecular protein population in human serum, the fine architecture of which has not been described before.

Materials and methods. Human serum was fractionated, starting with the removal of low density lipoproteins[1]. To the supernatant was added $(NH_4)_2SO_4$ solution, pH 7.2 (final concentration 1.5 *M*). The dissolved precipitate was filtered on a Bio-Rad A15 column and fractions eluting before or in the IgM region (Figure 1) were found to contain the macromolecules when examined by electron microscopy (EM). These fractions were concentrated, dialyzed and subjected to Pevicon electrophoresis. 3 fractions in the (α_1-) post albumin region were pooled and concentrated (Figure 2). Low-weight molecular contamination was eliminated on a Sephadex G200 column. The concentrated macromolecular fractions (0.2 mg protein/ml) were tested by immunodiffusion[2] against rabbit antiserum to whole human serum, C1, C3, C4, IgG, IgA,

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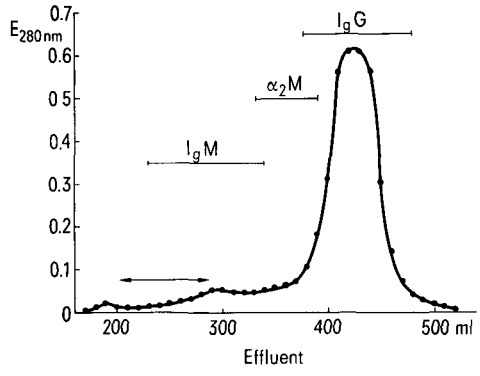

Fig. 1. Elution pattern of 5 ml of a partly purified serum preparation on a Bio-Gel A15 m column 180 × 1.9 cm, equilibrated with 0.05 *M* phosphate buffer pH 7.2 containing 0.2 *M* NaCl. The double arrow indicates the fractions which were pooled, concentrated and submitted to further purification.

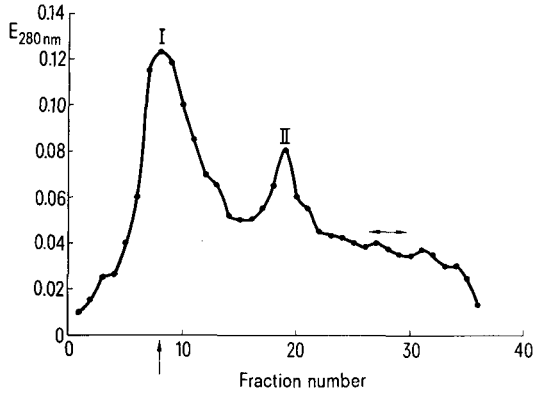

Fig. 2. Zone electrophoresis (Pevicon) of the macroglobulin containing material eluted from the Bio-Gel A15 m column (Figure 1). The arrow indicates the original site of application. The peaks under I and II contain IgM and α_2M respectively. Fractions marked by the double arrow were used for further studies.

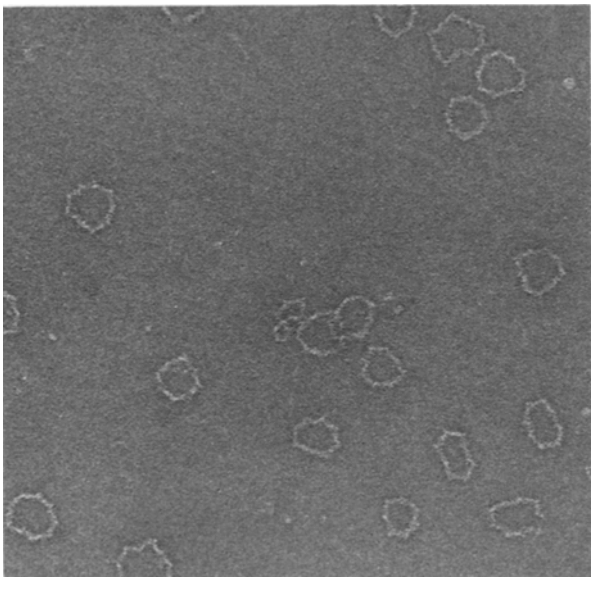

Fig. 3. Typical survey electron microscopic view of the protein macromolecules at rather low concentration × 140,000.

IgM, IgD, IgE, albumin, prealbumin, α_2M-globulin, transferrin, haptoglobin, ceruloplasmin, α_1-antitrypsin, fibrinogen, Gc-globulin and cholinesterase. The preparations were examined also by EM (Philips EM 200) using the negative contrasting technique.

The preparations were reduced by reacting 10 volumes of the purified material with 1 volume of 3 m*M* dithiothreitol, in 0.2 *M* *Tris*-HCl, pH 8.2, for 60 min at room temperature. For alkylation a 5-fold molar excess of iodoacetic acid in *Tris*-HCl buffer, pH 8.2 was used. Urea treatment was performed with 2, 4 or 6 *M* urea solutions for 1 to 8 h at room temperature. Samples were examined by EM at intervals of 1 h. Pepsin digestions were carried out in 0.2 *M* sodium acetate buffer, pH 4.4 for 24 h using an enzyme-substrate ratio of 2/100. The reaction was interrupted by adjusting the pH to 8.0. The density of the purified material was determined by the equilibrium centrifugation technique of MESELSON et al.[3]. Antigenic analysis of the material was attempted by the mixed haemadsorption technique, utilizing fixation of the soluble antigen on glass slides[4].

Results. Physicochemical properties. Elution chromatograms (Figure 1) suggested that the macromolecule has a molecular weight exceeding 1 million. The macromolecule migrated in the (α_1-) post albumin region and was resistant to reduction-alkylation under conditions which split human IgM into 7S subunits. Pepsin digestion caused no visible morphological alteration of the molecules, while human IgG molecules were split into $F(ab')_2$. The macromolecules disintegrated (EM) after a few h of storage in buffered 6 *M* urea but retained their morphology when stored at 4 °C over several weeks.

The proteinaceous character of the purified material was established by UV-absorption spectral analysis which showed high extinction values in the short wavelength (210–220 nm). Fractions from CsCl gradients were analyzed by EM and the characteristic macromolecules were banded at a density of 1.30 g/cm³.

Antigenic analyses. The sensitive mixed haemadsorption technique showed weak positive reactions for IgM, IgA and albumin with 3 preparations after $(NH_4)_2SO_4$-precipitation, chromatography and zone electrophoresis. The anti-albumin reaction disappeared after further fractionation of the preparations on Sephadex G 200.

Morphological features. The molecules were formed by multiplicity of a subunit with linear dimensions of 40–50 Å

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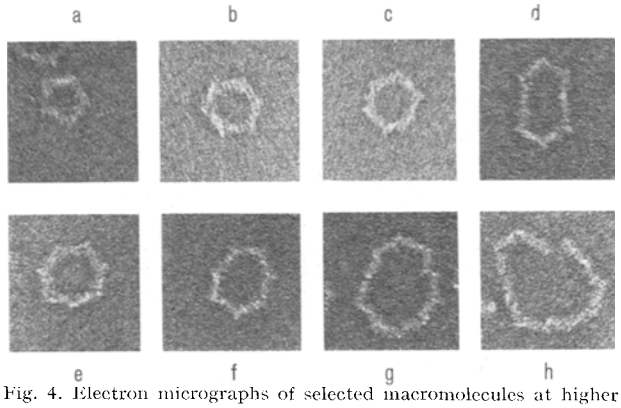

Fig. 4. Electron micrographs of selected macromolecules at higher magnification. a shows a pentagon, b–d) hexagons, e–f) heptagons, and g–h) larger less well-defined structures. The molecule in d) is basically an octagon since the length of 2 of the sides is twice that of the normal side piece. × 230,000.

×170 Å. This rodlike subunit forms one side in the mostly geometrically well-defined configurations of hexagons, heptagons and occasionally pentagons (Figures 3 and 4). Hexagons make up 25–30% of the material and heptagons 40–50%. Frequently also larger, less well-defined, structures with more than 7 subunits were seen (Figure 4). In some polymers, minor protrusions were visible at the corners where the subunits meet. In 12 preparations examined, macromolecules spontaneousyl ruptured in the corners were observed only on a few occasions (Figure 5a). Substructuration of the subunits was noted occasionally (Figure 5b). Two smaller beadlike subunits appeared to form the larger side piece in the polymer.

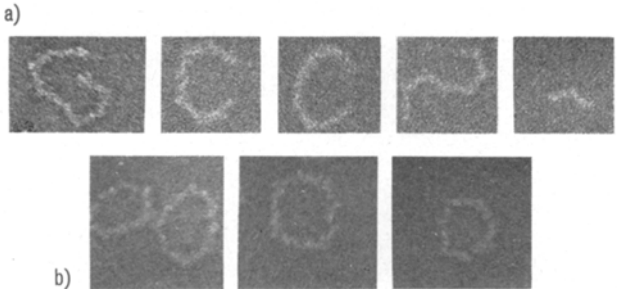

Fig. 5. a) Partially spontaneously disrupted molecules showing that the weak points are located at the corners. ×230,000. b) Grossly intact molecules with a substructuration of the subunits having the shape of beads or knobs. ×325,000.

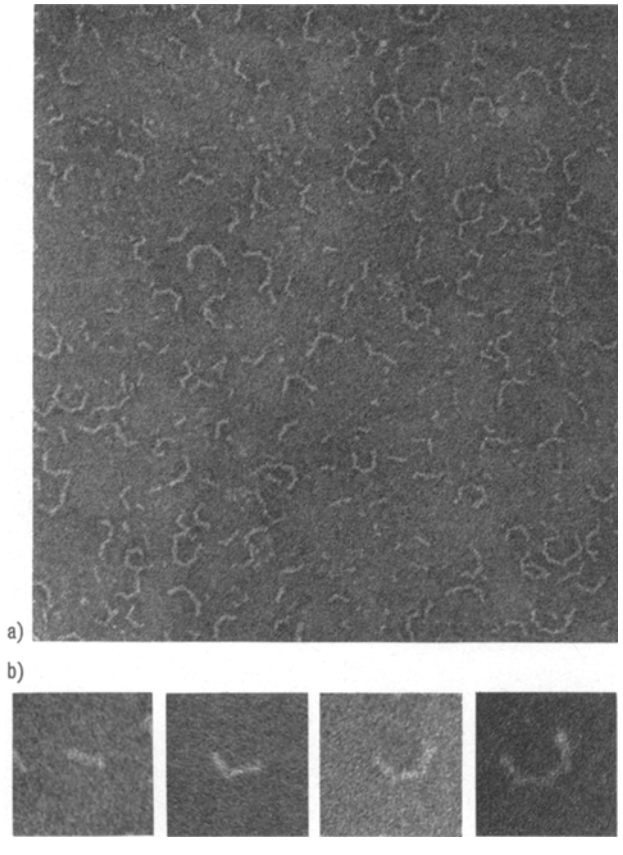

Fig. 6. a) Typical survey picture of macromolecules treated with 6 *M* urea for 2 h showing macromolecules in different stages of degradation. Note the presence of several single subunits. ×105,000. b) Degradation products (6 *M* urea) containing from 1 to 4 subunits. ×230,000.

After 2 h of treatment with 6 *M* urea, the macromolecules exhibited different stages of degradation from open polymers to single subunits (Figure 6).

Discussion. The ultrastructure of the protein molecule reported here has not, to our knowledge, been described before. Modifications of the purification procedure did not result in a loss of the macromolecules, although it affected the purity of the final product. Modifications included omission of the LDL-precipitation step, precipitation with 7% polyethylene glycol (MW, 6000) instead of $(NH_4)_2SO_4$, leaving out the electrophoretic separation etc. The characteristic macromolecular structure could be identified also after fractionation of human serum on a Bio-Rad A15 column.

The macromolecule is present in low concentrations (< 50 mg/l) in serum from healthy human donors, which may explain why it has passed unnoticed till now. It is impossible to exclude that the molecule represents a polymer of a known serum component, but our antigenic identification anaylses have been negative or inconclusive, since it could not be excluded that the weak positive reactions with anti-IgM and anti-IgA sera were due to trace contaminations. In addition, IgM molecules were observed occasionally to be enclosed by the polymer. Since the polymer migrates electrophoretically in the postalbumin region, it may be recalled that it has been difficult to raise precipitating antibodies to the 4.6 S-postalbumin protein[5]. Polymerism in connection with this protein is not described, however, and its biological function is unknown. Another component which deserves attention, as it is readily precipitable by 1.5 *M* $(NH_4)_2SO_4$ and has a molecular weight > 100,000, is the inter-α-trypsin-inhibitor. Further, isoenzymes, occurring in polymeric form, should be considered. Reduction-alkylation experiments and results of urea treatment indicate that the macromolecules are built up by subunits primarily linked to each other by non-covalent bonds. From the linear dimensions of the polymers and the weak reaction in the antigenic analyses, one could possibly make a case for IgA-albumin complexes[6], assuming that the corners of the polymer consist of Fc-parts, but neither the pepsin digestion data nor the spontaneous dissociation of the polymers at the juncture of the side pieces support this suggestion. Further studies of the biochemical and antigenic properties of the macromolecules may help to clarify their origin and biological function[7].

Zusammenfassung. EM-Analyse einer bisher nicht bekannten makromolekularen Serumfraktion. Das Makromolekül, präzipitierbar mit 1,5 *M* $(NH_4)_2SO_4$ und gereinigt durch Gel- Chromatographie und Elektrophorese, hat ein Molekulargewicht von ≧ 10^6 und wandert im postalbuminen Bereich.

B. BLOTH, G. UTTER and S.-E. SVEHAG[8]

Department of Immunology, Karolinska Institute Department of Medicine, Serafimerhospital and Department of Immunology, National Bacteriological Laboratory, Stockholm (Sweden), 21 January 1975.

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[7] The work was supported by the Swedish Medical Research Council (Project No. B 73-13X-2427-06A). The technical assistance of Miss RIGMOR TORSTENSSON is gratefully acknowledged.

[8] Present address: Institute of Medical Microbiology, Odense University, DK-5000 Odense, Denmark.