[CONTRIBUTION FROM THE LABORATORY OF PHYSICAL CHEMISTRY OF THE UNIVERSITY OF UPSALA]

THE MOLECULAR WEIGHT OF COCOSIN

BY BERTIL SJÖGREN AND ROMUALD SPYCHALSKI

RECEIVED JULY 5, 1930 PUBLISHED NOVEMBER 5, 1930

The oil-seed globulins, edestin,[1] amandin[2] and excelsin,[2] as well as the other vegetable proteins, legumin,[3] *r*-phycocyan,[4,5] *r*-phycoerythrin[4,5] and *c*-phycocyan,[5] so far studied in this Laboratory by means of ultracentrifugal analysis, have all proved to be monodisperse and to belong to the fourth group of proteins, having a molecular weight of about 208,000, that is, approximately six times the weight of ovalbumin.[6] They were found to be very stable, but with a tendency to dissociate into lower multiples of 34,500 at higher P_H values.[6]

In order to get more information concerning this large class of proteins, we took up the coconut globulin, in the following called cocosin,[7] for an ultracentrifugal examination. In chemical composition cocosin resembles excelsin very much, both having a high sulfur content, slightly exceeding 1%. With respect to solubility cocosin and edestin are almost identical, in so far as high salt concentrations are necessary to hold them in solution. Cocosin is obtained in a definite crystalline state, which may be taken as an indication of its homogeneity. Therefore the result arrived at was quite surprising: the protein was easily decomposed during the preparation and gave an unstable and polydisperse product.

Preparation of Material.—The nuts were freed from shells and skins, then ground in a mill to a fine paste (340 g.) and suspended in 10% sodium chloride solution (3000 cc.). Enough toluene was added to serve both as a preservative and as a solvent for the fat. The mixture was digested with stirring at room temperature for twenty-four hours. A test showed that no more protein could be extracted after this treatment. The bulk of the insoluble part was removed by means of a cloth filter. The solution was further freed from suspended particles and from oil and fat partly dissolved in the toluene by filtering and centrifuging. Saturated ammonium sulfate solution was then added to 60% saturation. After standing for one day at 5° the precipitate was filtered off and washed with half-saturated ammonium sulfate solution. The protein precipitate was dissolved in phosphate buffer of P_H 6.7 (0.033 *M* in KH_2PO_4 and 0.033 *M* in Na_2HPO_4), containing 7% of sodium chloride. This high P_H and salt content was used in order to increase the solubility. The solution was then dialyzed in the ice box for six days against the same buffer; volume of solution, 20 cc.; concentration, 3.05% (Ma-

[1] T. Svedberg and A. J. Stamm, THIS JOURNAL, **51**, 2170 (1929).

[2] T. Svedberg and B. Sjögren, *ibid.*, **52**, 279 (1930).

[3] T. Svedberg and B. Sjögren, *ibid.*, **52**, 3279 (1930).

[4] T. Svedberg and N. B. Lewis, *ibid.*, **50**, 525 (1928).

[5] T. Svedberg and T. Katsurai, *ibid.*, **51**, 3573 (1929).

[6] T. Svedberg, *Kolloid-Z.*, **51**, 10 (1930).

[7] Compare T. B. Osborne, "Vegetable Proteins," Longmans, Green and Co., London, **1916**, pp. 57, 78.

terial I). The substance was comparatively homogeneous, consisting principally of one component.

It may be mentioned that the above method of preparation was used for preparing another batch of cocosin, the final product, however, being dissolved in 10% sodium chloride solution. This material was very non-homogeneous, breaking up more and more with time. The same result was obtained when coconut meal was used instead of the fresh nuts.

Before carrying out these preparations we also made an attempt to get the protein in a pure state by dialyzing the solution against water after precipitation with ammonium sulfate and dissolving in 10% sodium chloride. When working with proteins we have often had the opportunity to observe that some of them, and especially the oil-seed globulin, are very sensitive to long water dialysis. Hydrolysis and denaturation then occur. Cocosin showed this phenomenon in a pronounced way, the crystallized cocosin material prepared by dialysis against water proving to be very difficult to bring into solution. By centrifugal analysis this material was found to be quite decomposed.

To avoid these difficulties, especially the using of high salt contents, which complicates the centrifugal analysis, we modified the method in the following way. After precipitation with crystallized ammonium sulfate to 60% saturation, the globulin was dissolved in 5% sodium chloride solution. Hereafter, in order to increase the solubility, Na_2HPO_4 was added to a concentration of 0.017 M. Then, before a second precipitation, the P_H of the solution was decreased to 5.5 by means of KH_2PO_4. This brought about no change in the precipitation limits. After treatment as before, cocosin was completely dissolved in Na_2HPO_4 (0.2 M) and dialyzed against the same solvent for six days. From 700 g. of coconut paste we derived 30 cc. of solution containing 0.99% cocosin (Material II). The protein was found to be a mixture of two components.

In our last attempt to prepare cocosin we worked as quickly as possible, also using the simplest method of purification. After digesting the paste (340 g.) and precipitating once as in the case of Material I, the product was dissolved in phosphate buffer of P_H 6.7 (0.033 M in KH_2PO_4 and 0.033 M in Na_2HPO_4), containing also 4% of sodium chloride, and dialyzed against the same buffer in the ice box for five days; volume of solution, 50 cc.; concentration, 0.74% (Material III). This substance was also a mixture of two molecular species.

Specific Volume and Light Absorption.—For both these measurements Material III was used at a P_H of 6.7 with solvent as above.

The partial specific volume was determined pycnometrically at 20.2°. Two determinations at different concentrations gave the mean value 0.746 in agreement with the values obtained for the other proteins which follow the law of simple multiples.

The light absorption was studied by means of the Judd-Lewis spectrophotometer as described in earlier communications. The protein concentrations were 0.15 and 0.074% with a thickness of layer 2.0 cm. The absorption maximum $\epsilon/c = 7.0$ was situated at 280 mμ, and the minimum $\epsilon/c = 2.7$ at 255 mμ. The absorption curve is given in Fig. 1.

Determination of Sedimentation Constant and Different Molecular Components.—The runs were carried out with the high-speed oil-turbine ultracentrifuge. In the case of Material I the speed was 23,000, in the other runs about 43,000, r. p. m. The time varied from two to three and one-half

hours, the temperature during each run from 20 to 23°, other experimental conditions being as usual.

The sedimentation constant is given by $s = (dx/dt) \times 1/(\omega^2 x)$, where x is the distance from axis of rotation, ω the angular velocity and t the time. In cases of non-homogeneous material the amounts of the different molecular species may be calculated from their sedimentation and diffusion constants, reducing the galvanometer deflections of the microphotometer curves to represent relative concentrations.[1,2,4] The centrifugal force must be large enough to give a marked separation of the components. Thus, at the highest possible speed, *viz.*, 45,000 r. p. m., giving a centrifugal force of about 120,000 times that of gravity, it is easy to observe a component of weight 6 × 34,500 in mixture with a substance of molecular weight 3, 2 or 1 × 34,500. It is also possible to distinguish a component of 104,000 from one of 34,500 but not from one of 68,000, without using a very long time of centrifuging. Owing to the difficulties of taking into account the difference in light absorption between the normal and the decomposed product, the calculations of the relative amounts of the components should only be regarded as an approximation.

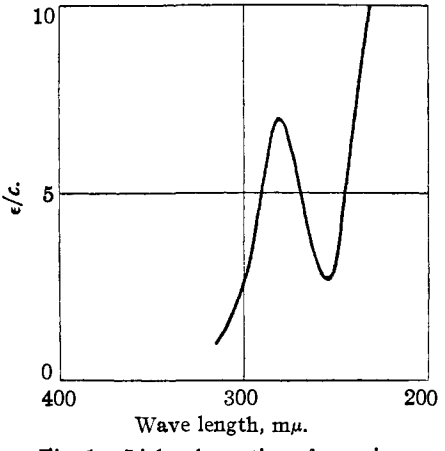

Fig. 1.—Light absorption of cocosin.

In Table I the determinations with the Materials I, II and III are summarized. The other preparations gave such unstable products that we refrain from mentioning the values obtained.

TABLE I

COCOSIN, SUMMARY OF SEDIMENTATION VELOCITY MEASUREMENTS

Subs.	Solvent KH_2PO_4, *M*	Solvent Na_2HPO_4, *M*	Solvent NaCl, %	P_H of soln.	Concn. of protein	$s_{20°} \times 10^{13}$	State of soln.
Material I	0.033	0.033	4.7	6.7	0.30	10.47	[a]
						corr. value 12.4	
Material I	.013	.053	2.4	7.4	1.30	11.37	[a]
Material II	..	.200	...	7.9	0.20	...	[c]
Material III	.033	.033	4.0	6.7	.37	...	[d]
Material III	.030	.030	3.6[a]	9.7	.13	...	[e]
Material III	.015	.035	...[b]	11.8	.37	9.71	[f]

[a] 0.033 *M* in NaOH. [b] 0.067 *M* in NaOH. [c] Mixture of approximately 65% molecules of weight 208,000 and 35% of weight 104,000. [d] Mixture of approx. 75% 208.000, 25% 104.000. [e] Mixture of approximately 50% 208,000, 50% 104,000. [f] Decomposed; 15% non-centrifugible products.

As may be seen from the table, Material I was almost homogeneous, giving a value of s quite in agreement with the values obtained for the other proteins possessing a molecular weight of 208,000. A small amount of a lighter component was present.

There were at least two molecular species present in Material II. The stock solution was a mixture of about 65% molecules of weight 208,000 and 35% of weight 104,000. Assuming the cocosin molecule to be of weight 208,000, then the presence of the smaller component can be considered as the first stage of the protein cleavage, caused by the high P_H. This splitting-up of the molecule with increasing P_H has in earlier cases, as a rule, been found to be reversible. Thus if the P_H of the solution is brought back to a value lying in the stability region, one would expect a monodisperse product. The stock solution of Material II was dialyzed against a phosphate buffer of P_H 6.0 (0.17 M in KH_2PO_4 and 0.03 M in Na_2HPO_4, containing 4% of sodium chloride) for five days. By a second analysis we found that the amount of the smaller component had decreased. In order to complete the reaction in this direction the dialysis was continued for six days more. During this treatment the cocosin was, however, totally decomposed which behavior is in line with the former experience on the high sensitivity of the oil-seed globulins to prolonged dialysis.

Material III proved to be somewhat more homogeneous in so far as the stock solution had approximately a composition of 75% molecules of weight 208,000 and 25% of weight 104,000. In more alkaline solutions the protein was broken up into units of one-half the normal molecular weight and at still higher P_H values into particles of unequal size. A run was also made at a P_H of 5.5, using phosphate buffer and 9% sodium chloride, which confirmed the result obtained at P_H 6.7. Because of the very high salt content, involving great corrections, the values are not reproduced here.

Determination of Molecular Weight.—For the sedimentation–equilibrium method, the molecular weight is given by the relation

$$M = \frac{2RT \ln (c_2/c_1)}{(1 - V\rho)\omega^2(x_2^2 - x_1^2)}$$

where c_1 and c_2 are the concentrations at the distances x_1 and x_2 from the center of rotation, R the gas constant, T the absolute temperature, V the partial specific volume of the solute, ρ the density of the solvent and ω the angular velocity.

Two runs were made with Material III at P_H 6.7 using the same buffer as before and the concentration 0.15%. In Fig. 2 the values of the molecular weight are plotted against the distance from the center of rotation. As may be seen from the diagram the material was non-homogeneous with regard to molecular weight, giving values from approximately 215,000 down to 110,000 with the higher values all lying around 208,000. As the

time between the two runs was nearly a week, the slight difference observed may be interpreted as a further splitting-up of the cocosin.

The results of our measurements make it extremely probable that normal native cocosin has a molecular weight of the same magnitude as the other oil-seed globulins so far analyzed, *viz.*, six times the weight of ovalbumin or 208,000. Their molecules are all spherical with a molecular radius of about 3.95 mμ. The component with a molecular weight half that of the normal present in the cocosin samples studied may be considered as the first decomposition product of the protein cleavage.

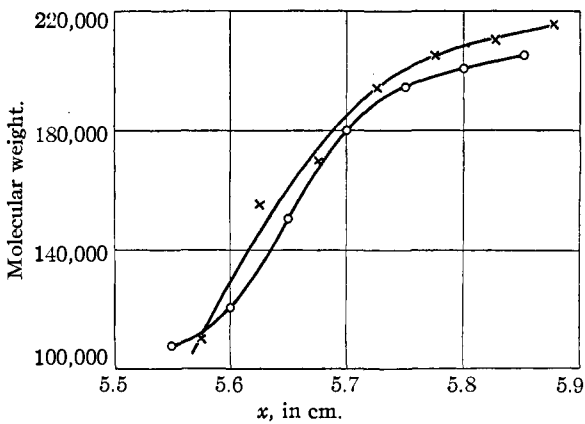

Fig. 2.—Variation of molecular weight with distance from axis of rotation: —×—, Run 1; —○— Run 2, one week later.

The expenses connected with this work have been defrayed by a grant from the foundation "Therese och Johan Anderssons Minne."

Summary

1. By means of ultracentrifugal methods an attempt was made to determine the molecular weight of the coconut globulin, called cocosin.

2. This protein was found to be rather unstable, being easily decomposed during the process of preparation.

3. The centrifugal analysis proved cocosin to be a mixture of two main components of weight about 208,000 and 104,000 with the larger molecules present in much greater amounts than the smaller ones.

4. It was considered as extremely probable that native cocosin has a molecular weight within experimental limits identical with the other oil-seed globulins earlier studied, *viz.*, 208,000, and that the small fraction present of weight half of this normal molecule represents the first stage of the protein cleavage.

UPSALA, SWEDEN