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

THE MOLECULAR WEIGHTS OF PHYCOCYAN AND OF PHYCOERYTHRIN. III

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In two previous papers[1,2] reports have been given of the determination of the molecular weights and the sedimentation constants of phycocyan from *Ceramium rubrum*, *Porphyra tenera* and *Aphanizomenon flos aquae* and of phycoerythrin from *Ceramium rubrum* and *Porphyra tenera*. In the present paper some new data concerning these interesting proteins will be communicated.

For some of the sedimentation velocity measurements a new high-speed ultracentrifuge giving a centrifugal force 200,000 times gravity at the center of the cell has been used. By the aid of this powerful machinery it was found possible to analyze *r*-phycocyan in the P_H region 5.5–6.7 where the sedimentation constant as measured at lower speed changes rapidly with the P_H and to show that *r*-phycocyan in this region actually consists of a mixture of normal molecules and half-molecules (see Figs. 8 and 9).

The new ultracentrifuge is constructed on the same general principles as the previous one which gave a centrifugal force 100,000 times gravity at a speed of 42,000 r. p. m. with an effective radius of 52 mm.[3] In order to attain a doubling of the centrifugal force, both the effective radius and the speed have been increased. The first rotor used in the present work was cylindrical, like the one described previously, and had a diameter of 180 mm. and a thickness of 60 mm.[3] It was provided with two holes for cells, 34 mm. in diameter with their centers 65 mm. from the center of the rotor. In order to lessen the strain in the rotor at high speed, part of the material between the cell holes was removed by drilling a number of extra holes through the rotor and closing them with steel plates (to prevent false light from reaching the photographic plate). This rotor exploded at a speed of 50,000 r. p. m. during a sedimentation velocity determination on phycoerythrin, although it had already been run at 55,000 r. p. m. several times. A new rotor of the same diameter, but only 54 mm. in thickness and with cell holes only 30 mm. in diameter, was then made. This rotor was tested at a speed of 63,000 r. p. m. corresponding to a centrifugal force 285,000 times gravity at the center of the cell and has since then been run regularly at speeds up to 55,000 r. p. m., which gives 218,000 times gravity. It was found rather difficult to construct cells which

[1] Svedberg and Lewis, THIS JOURNAL, **50**, 525 (1928).

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

[3] Svedberg and Lysholm, *Nova Acta Reg. Soc. Scient. Upsaliensis*, vol. ex. ord., ed. 1927: Svedberg and Nichols, THIS JOURNAL, **49**, 2920 (1927).

would withstand these enormous centrifugal forces but this trouble has been overcome. The limit of the centrifugal force attainable is set by the breaking limit of the material of the rotor.

It is possible to lessen the strain in the rotor still more by machining away more of the material between the cell holes so as to give the rotor an

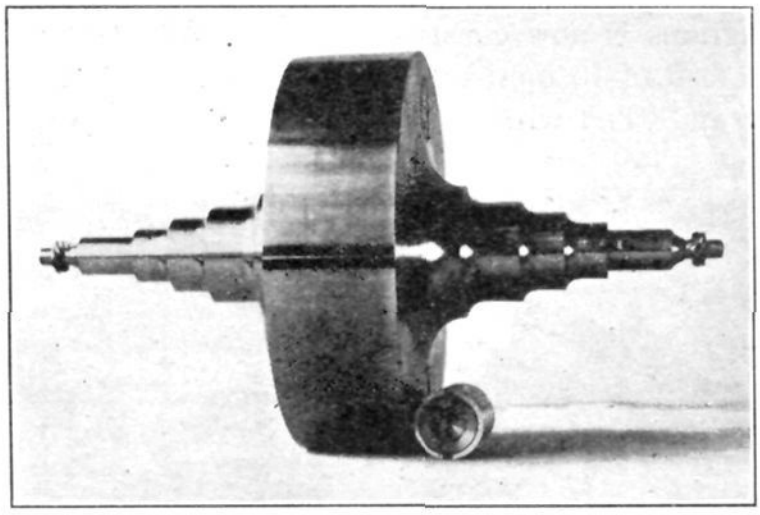

Fig. 1.

oval shape with a circular disk left for screening off false light. A rotor of this shape with an effective radius of 50 mm. has been run in the old ultracentrifuge with very good results at speeds up to 55,000 r. p. m., corresponding to a centrifugal force 170,000 times gravity. An oval rotor is now being made for the new ultracentrifuge and it is hoped that it will be possible to run it regularly at speeds up to 65,000 r. p. m. which will give a centrifugal force 300,000 times gravity at the center of the cell.

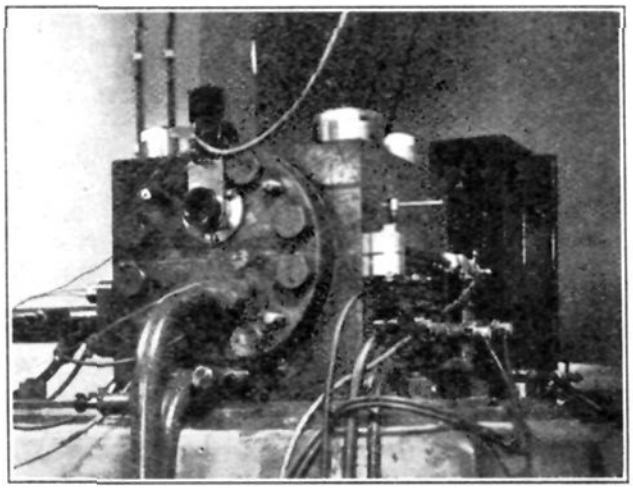

Fig. 2.

Figure 1 shows the rotor and Fig. 2 the centrifuge used for the sedimentation analysis of *r*-phycocyan. In Fig. 3 a general view of the apparatus is given. A detailed description of the new ultracentrifuge will be published elsewhere.

For converting the microphotometric records of the sedimentation pictures into diagrams giving the relation between concentration and distance from the center of rotation, a slightly different method has been adopted both for the sedimentation equilibrium and the sedimentation

velocity measurements. Previously a concentration scale was photographed onto the plate carrying the sedimentation pictures by means of filling the cell in turn with a series of different concentrations of the solution studied, speeding up the centrifuge and taking pictures with the same times of exposure as for the sedimentation pictures. This procedure is rather tedious. The intensity scale necessary for evaluating the sedimentation diagrams is now constructed in the following way. A plane parallel quartz cell of 10 mm. thickness is placed between the lamp and the centrifuge and filled with the solvent used for the solution which is

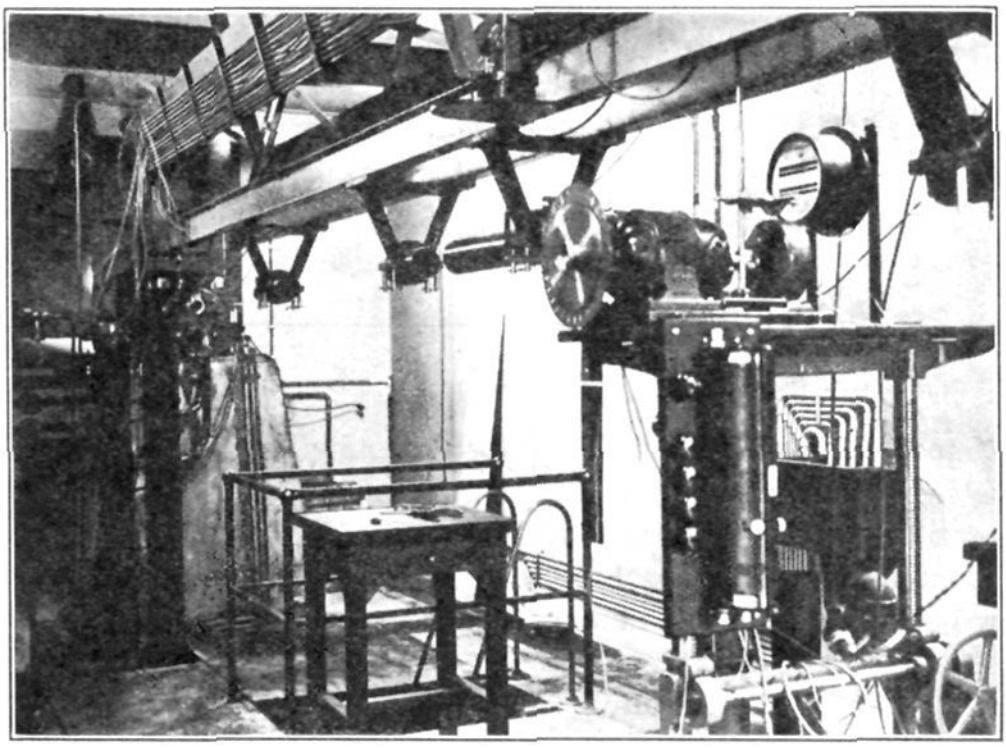

Fig. 3.

being centrifuged. In the case of a sedimentation velocity determination the following procedure is used. At the end of the run when a layer of pure solvent has been formed at the top of the column of solution the cell outside the centrifuge is filled in turn with a series of different concentrations of a suitable arbitrary solution, say, potassium chromate, and pictures are taken on the plate which carries the sedimentation pictures. A curve giving the relation between concentration of the arbitrary solution and blackening is then constructed, as well as a curve giving the relation between distance from the center of rotation and blackening in the sedimentation picture. It is easily seen that for pairs of points in the two curves corresponding to equal blackenings the ratio of the concentrations must be equal. The relative concentrations in the sedimentation picture corresponding to the different distances from the center of rotation can

therefore be found from the first curve. The only assumption which has been made is that the light absorption constant in both the arbitrary solution and the solution centrifuged is proportional to the concentration. This condition is fulfilled for the solutions studied so far.

In the case of a sedimentation equilibrium measurement where pure solvent is not formed at the top of the column of solution, the cell rotating in the centrifuge has to be filled with pure solvent when the pictures of the arbitrary solution are taken.

The Sedimentation Constant of Native Phycocyan from Aphanizomenon Flos Aquae.—In the course of the ultracentifugal investigations on proteins carried out in this Laboratory, it has become evident that there exist cases where the so-called purified protein is more or less an artificial laboratory condensation product built up out of some low molecular native substance through the action of the chemicals used in the purification process, especially the ammonium sulfate. It therefore seemed of considerable importance to reëxamine phycocyan and phycoerythrin in a state as close to their native occurrence as possible.

The *Aphanizomenon* material was collected in Lake Valloxen near Upsala. It was freed from excess of water by centrifuging and kept at room temperature for three days to allow autolysis to set in. After that time the aqueous extract was filtered off and clarified in a Sharples centrifuge. Toluene was added as a preservative. Table I gives the determinations of the sedimentation constant.

TABLE I

AQUEOUS EXTRACT OF APHANIZOMENON CONTAINING *C*-PHYCOCYAN, SEDIMENTATION VELOCITY MEASUREMENTS

Centrifugal force 200,000 to 210,000 times gravity; thickness of column of soln., 1.20 cm.; source of light, mercury lamp; light filter, Wratten K3; aperture of lens F:25; plates, Ilford rapid process panchromatic; time of exposure 10 sec.; metol developer, 1 min.

Solution	$S_{20°} \times 10^{13}$
Extract diluted 10 times with 1% NaCl	11.6
Extract diluted 4 times with 1% NaCl	11.7
Extract diluted 4 times with 1% NaCl	11.2
	Mean value 11.5

The sodium chloride was added in order to depress the Donnan effect. Previous experience with native lactalbumin[4] and native ovalbumin[5] has shown that sodium chloride probably does not affect the molecular state of native proteins. For the sedimentation constant of *c*-phycocyan purified by crystallization with ammonium sulfate, a value of 11.2×10^{-13} has been found.[2] These values are identical within the limits of experi-

[4] Sjögren and Svedberg, THIS JOURNAL, **52**, 3650 (1930).

[5] Unpublished determinations by Sjögren; *cf. Nature*, **128**, 999 (1931).

mental error, proving that *c*-phycocyan exists in the cell extract in the same molecular state as in the solutions of the purified protein.

The Sedimentation Constant of Native Phycoerythrin from Ceramium Rubrum.—The *Ceramium* material was collected near Havstensund on the west coast of Sweden. It was washed six times with sea water in order to remove contaminations, then divided into three parts and treated in the following way. (1) One part of the alga plus five parts of distilled water was kept at room temperature for three days; the extract was filtered off and examined in the ultracentrifuge without the addition of toluene. (2) The material was treated as above but with only four parts of distilled water to one part of alga and toluene was added to the extract after three days. (3) The washed material was kept in sea water for one day, after which time phycoerythrin began to appear in the water; the mass was squeezed out and left with five parts of sea water for three days, after which time the extract was filtered off and toluene was added. Table II gives the determinations of the sedimentation constant.

TABLE II

EXTRACT OF CERAMIUM CONTAINING *r*-PHYCOERYTHRIN, SEDIMENTATION VELOCITY MEASUREMENTS

Centrifugal force 100,000 times gravity; optical conditions similar to those of Table I

Solution	$S_{20°} \times 10^{13}$
Extract 1 diluted 4 times with 1% NaCl	11.5
Extract 2 diluted 3 times with 1% NaCl ($P_H = 7$)	11.1
Extract 2 diluted 4 times with 1% NaCl	11.8
Extract 2 run through the Sharples centrifuge and then diluted 4 times with 1% NaCl	11.3
Extract 3 diluted 3 times with 1% NaCl	11.3
Mean value	11.5

The sedimentation constants for the three extracts are identical within the limits of error and also identical with the value for purified *r*-phycoerythrin, *viz.*, 11.5×10^{-13}, as previously determined.[1,2] The amount of *r*-phycocyan present in Extracts 1 and 2 is too small compared with the amount of phycoerythrin to be noticeable in the sedimentation diagrams. In Extract 3 there was no phycocyan at all because when treating the alga with sea water all the phycocyan and some of the phycoerythrin remains in the cells and is not extracted. The run on Extract 1 compared with the runs on Extract 2 show that toluene has no effect on the molecular state of phycoerythrin. The run on Extract 3 shows that the salts of the sea water do not influence the sedimentation constants of this protein.

The Sedimentation Constant and Molecular Weight of Phycoerythrin from Polysiphonia Urceolata.—During his investigations on the occurrence of phycocyan and phycoerythrin in various algae, Kylin came across a new modification of phycoerythrin in a material of *Polysiphonia* collected

near Kristineberg on the west coast of Sweden. It is closely allied to the form of *r*-phycoerythrin which occurs in *Ceramium* and *Porphyra*;[6,7] the light-absorption curve[7] shows three maxima in the visible corresponding very closely to the maxima of normal phycoerythrin except that the maximum in the blue-green is the strongest one in this variety, while it is the weakest one in normal phycoerythrin.[8] The most marked difference in optical respect between the two forms is, however, the very slight fluorescence of the *Polysiphonia* phycoerythrin compared with that of the normal form. Professor Kylin has kindly put at our disposal the phycoerythrin material which he had extracted from *Polysiphonia urceolata* in the summer of 1930 and which had been purified by precipitating five times with ammonium sulfate. The ultracentrifugal measurements were carried out in the fall of 1931, the solution having been kept in the refrigerator at a temperature between 0 and 4° in the meantime. The sedimentation constant was measured at different P_H values and the molecular weight was determined near the isoelectric point by means of the sedimentation equilibrium method.

The material proved to be inhomogeneous with regard to molecular weight. Values ranging from 160,000 to 270,000 were found. The mean weight was 196,000, which shows that the main constituent of the material probably possesses the same molecular weight, 208,000, as the ordinary phycoerythrin. Because of the inhomogeneity of the material, much stress cannot be put on the values of the sedimentation constant obtained. Within the P_H range 4.4–8.0 the sedimentation velocity was found to be independent of P_H. Ten determinations gave for the sedimentation constant 12.4×10^{-13}, which value is about 10% higher than that found for the ordinary phycoerythrin. The discrepancy is probably due to the inhomogeneity of the material. At a P_H of 10.6, the protein was partly decomposed. The *Polysiphonia* phycoerythrin therefore resembles closely the ordinary phycoerythrin.

The Sedimentation Constant of Phycoerythrin from Bornetia Secundiflora, Griffithsia Furcellata and Sebdenia Monardiana.—During a stay at the Stazione Zoologica at Naples, Italy, one of us (T. S.) prepared extracts of some Mediterranean algae in order to see whether any phycoerythrin or phycocyan of abnormal behavior could be found there.[9] To judge

[6] Kylin, *Z. physiol. Chem.*, **76**, 396 (1912).

[7] Kylin, *ibid.*, **197**, 1 (1931).

[8] The light absorption curve was measured in this Laboratory by Dr. A. Tiselius and published by Kylin. The discrepancy between the values for the wave lengths of the absorption maxima of phycoerythrin and phycocyan as given by Svedberg and Katsurai[2] and those of Lemberg [*Ann. Chem.*, **461**, 46 (1928)] was found by Tiselius to be due to a change in the zero-point of the instrument used by Katsurai.

[9] For the kindness and help shown him on this occasion by the Director, Prof. R. Dohrn, as well as by the Staff of the station, sincere thanks are expressed.

from the color and fluorescence of the extracts three of the algae examined, *viz.*, *Bornetia secundiflora*, *Griffithsia furcellata* and *Sebdenia monardiana* seemed to be of special interest and the study of these extracts was therefore continued in Upsala.

The extracts were precipitated with 20 g. of ammonium sulfate per 100 cc., the precipitates were dissolved in water and the solutions reprecipitated with 25 g. of ammonium sulfate per 100 cc. These precipitates were then dissolved in water and dialyzed in the cold for a week against 0.001 *M* phosphate buffer of P_H 6.8. Sedimentation velocity determinations were carried out within the P_H range 5–9. No marked change of the sedimentation constant with P_H was found in this region. Two determinations on *Bornetia* phycoerythrin gave 12.1×10^{-13}, four determinations on *Griffithsia* phycoerythrin 12.1×10^{-13} and three determinations on *Sebdenia* phycoerythrin (one of which was carried out on the crude extract) gave 11.3×10^{-13}. The value for the last kind of phycoerythrin is identical with the values obtained for phycoerythrin from *Ceramium* and from *Porphyra*. The values for the *Bornetia* and the *Griffithsia* phycoerythrin are slightly higher and identical with the value found for phycoerythrin from *Polysiphonia*. These higher values are probably due to inhomogeneity of the material.

The phycoerythrin modifications contained in *Bornetia*, *Griffithsia* and *Sebdenia* therefore resemble ordinary *r*-phycoerythrin both with regard to sedimentation constant and P_H stability. To judge from the color, the phycoerythrin from *Bornetia* seems to be identical with the ordinary phycoerythrin from *Ceramium* and from *Porphyra*. *Griffithsia* phycoerythrin, however, is more purple-red in color and has a much weaker fluorescence. *Sebdenia* phycoerythrin is of a light red color and has a very strong yellow fluorescence. In order to have a quantitative comparison of their color, the light absorption bands in the visible spectrum for the phycoerythrin from *Bornetia*, *Griffithsia* and *Sebdenia* were determined by means of the König–Martens spectrophotometer.[10] The extinction coefficients as a function of wave length are given in the diagrams, Figs. 4, 5 and 6, which also contain

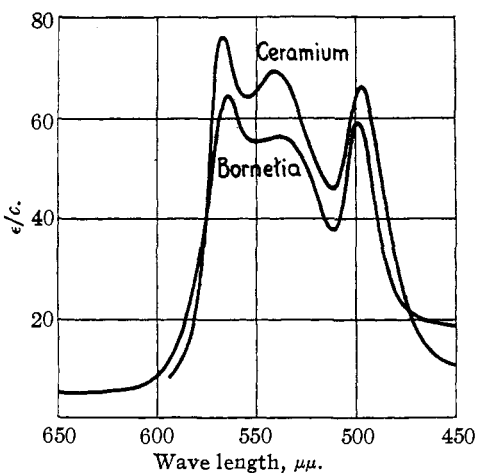

Fig. 4.

[10] We are indebted to Mr. S. Brohult for carrying out these measurements.

the curves for ordinary *Ceramium* phycoerythrin and for *Polysiphonia* phycoerythrin. It follows from the diagrams that the light absorption is very similar for ordinary phycoerythrin and *Bornetia* phycoerythrin on the one hand and for *Griffithsia* and *Polysiphonia* phycoerythrin on the other. *Sebdenia* phycoerythrin differs from all the others by having only two instead of three bands, the middle band being absent. This fact has already been noticed by Gaetano[11] in his extensive qualitative study of the light absorption of extracts from Mediterranean algae.

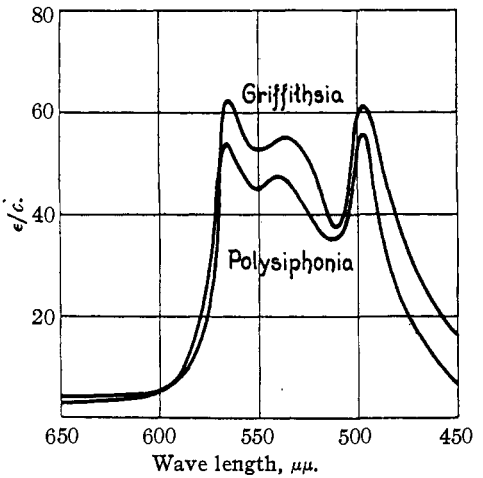

Fig. 5.

It seemed desirable also to get at least a semi-quantitative test of the difference in fluorescence between the phycoerythrin modifications. Dr. A. Tiselius of this Laboratory kindly undertook such an investigation, using an apparatus for Raman spectrum measurements. No definite difference in wave length of the fluorescence light was found for phycoerythrin from *Ceramium*, *Polysiphonia*, *Bornetia*, *Griffithsia* and *Sebdenia*. The intensity, however, was distinctly lower in the case of *Polysiphonia* and *Griffithsia*. No trace of the phycocyan fluorescence could be detected in the solutions obtained from *Polysiphonia*, *Bornetia*, *Griffithsia* and *Sebdenia*, although owing to the scarcity of the material at our disposal no attempt had been made to remove any phycocyan present. A comparison with the raw product of phycoerythrin from *Ceramium* showed that in this case phycocyan shows up distinctly in the fluorescence spectrum. It therefore appears that practically no phycocyan is present in *Polysiphonia*, *Bornetia*, *Griffithsia* and *Sebdenia*. The one which, to judge from the color of the solution, seemed most likely to contain traces of phycocyan was the *Griffithsia* solu-

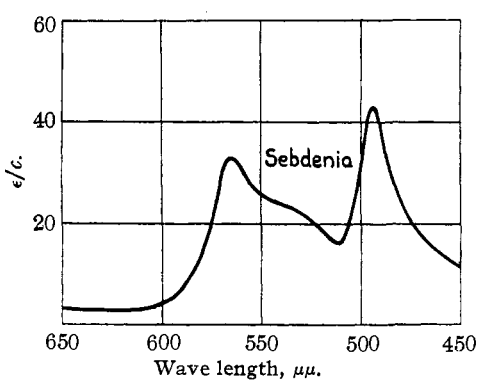

Fig. 6.

[11] Gaetano, *Publ. Staz. Zool. Napoli*, **7**, 88 (1926).

tion. It was therefore submitted to a still more severe test, electrophoretic analysis. Owing to the difference in isoelectric points phycoerythrin and phycocyan may easily be separated in a suitable moving boundary apparatus as shown by Tiselius.[12] The test, which was carried out by Dr. K. O. Pedersen of this Laboratory, proved that no phycocyan was present.

The Sedimentation Constant and Molecular Weight of *r*-Phycocyan from Ceramium Rubrum.—In a previous communication[2] it was shown that *r*-phycocyan from *Porphyra tenera* has a molecular weight of 206,000 at P_H 4.6 but only about half this weight, *viz.*, 107,000 at P_H 6.8. The sedimentation constant, which was found to be 11.1×10^{-13} at P_H 4.6, dropped to 5.98×10^{-13} at P_H 6.8. For *r*-phycocyan from *Ceramium rubrum*[1] a molecular weight of 106,000 had been found at P_H 6.8–7.9 with a sedimentation constant at P_H 6.8 of 5.59×10^{-13} (value reduced to 20°). In view of the fact that no determinations exist for *r*-phycocyan from *Ceramium* at P_H values where one would expect the higher molecular weight and higher sedimentation constant, it seemed desirable to carry out such determinations and at the same time make a complete study of the P_H stability curve for this interesting protein.

The *r*-phycocyan used in the present investigation was prepared in the following way. To 7500 g. of *Ceramium* which had been washed with sea water and superficially dried by pressing was added 2830 cc. of distilled water. After standing for twenty-four hours at room temperature, 10 cc. of toluene was added and after two more days the extract was filtered off. The 3650 cc. of liquid obtained was precipitated with 20 g. of ammonium sulfate per 100 cc. After standing for two days the precipitate was centrifuged off in a Sharples centrifuge and dissolved in 900 cc. of water. This solution was clarified by centrifuging and filtering. The separation of phycocyan from phycoerythrin was carried out as follows. To the solution were gradually added small amounts of saturated ammonium sulfate solution with intervals of three days according to the following scheme: 10 + 2.5 + 2.5 + 2.5 + 2.5 + 2.5 g. of salt per 100 cc. The precipitate was centrifuged off after every addition of ammonium sulfate and mixed with a small quantity of water. Most of the phycoerythrin and some phycocyan go into solution, while the bulk of the latter protein remains undissolved. Thus two fractions were obtained, *viz.*, the solutions on the one hand and the crystals on the other. The latter were brought into solution again and the two resultant solutions fractionated once more in the same way. If the process of dissolution is carefully controlled by observations in the microscope, it is possible to obtain pure phycocyan after the second series of fractionations. In order to dissolve the crystals completely a trace of Na_2HPO_4 had to be added. The P_H was then lowered by adding KH_2PO_4. The final phycocyan solution was

[12] Tiselius, *Nova Acta Reg. Soc. Scient. Upsaliensis*, [4] **7**, No. 4 (1930).

dialyzed for a week in the cold. The yield was 40 cc. of a 0.44% phycocyan solution.

TABLE III

r-PHYCOCYAN FROM CERAMIUM, SEDIMENTATION VELOCITY MEASUREMENTS

Centrifugal force about 100,000 times gravity; thickness of column of soln., 1.2 cm.; source of light, mercury lamp; light filter, nickel oxide glass (first and third run, Wratten K3); plates, Imperial process (first and third run, Ilford rapid process panchromatic); time of exposure, 20 sec.; metol hydroquinone developer, 1 min. (first and third runs, metol developer, 1 min.)

Solvent				P_H of soln.	Concn. of protein, %	$S_{20} \times 10^{13}$
	M		*M*			
HCl	0.10	.....	..	1.0	0.04	8.0
HCl	.007	.....	..	1.3	.07	7.1
HCl	.03	KCl	0.02	1.5	.04	5.9
HCl	.03	KCl	.03	1.6	.09	5 9
HCl	.03	KCl	.03	1.6	.08	5.4
HCl	.02	KCl	.03	1.8	.10	5.9
HCl	.01	KCl	.04	2.0	.08	10.5
HCl	.007	KCl	.04	2.5	.07	11.4
HCl	.001	KCl	.04	3.0	.07	11.4
HAc[a]	.019	NaAc	.001	3.5	.07	10.6
HAc[a]	.016	NaAc	.004	4.0	.09	11.2
HAc[a]	.006	NaAc	.014	5.0	.10	11.7
HAc[a]	.006	NaAc	.014	5.0	.10	11.4
KH_2PO_4	.095	Na_2HPO_4	.005	5.5	.09	10.4
KH_2PO_4	.090	Na_2HPO_4	.010	5.8	.09	10.4
KH_2PO_4	.080	Na_2HPO_4	.020	6.2	.07	9.4
KH_2PO_4	.070	Na_2HPO_4	.030	6.5	.09	7.9
KH_2PO_4	.050	Na_2HPO_4	.050	6.8	.09	5.8
KH_2PO_4	.030	Na_2HPO_4	.070	7.1	.09	6.2
KH_2PO_4	.020	Na_2HPO_4	.080	7.3	.09	5.8
KH_2PO_4	.010	Na_2HPO_4	.090	7.7	.08	6.1
KH_2PO_4[a]	.005	Na_2HPO_4	.095	8.0	.09	5.7
.....	..	Na_2HPO_4	.10	8.6	.09	5.1
NaOH	.001	Na_2HPO_4	.009	9.1	.09	3.7

[a] 1% in NaCl.

The sedimentation constant was studied in the P_H range 1–10.5. In Table III and Fig. 7 the determinations are summarized. At a P_H higher than 9.1 the values of the sedimentation constant were erratic and uncertain owing to the decomposition of the molecule. These figures have therefore been omitted.

From Table III and Fig. 7 it follows that there are seven regions with regard

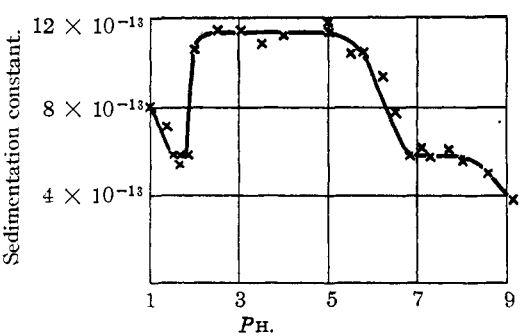

Fig. 7.

to the relation between sedimentation constant and P_H. The middle one is the widest. It extends from P_H 2.5 to 5.0 and contains the isoelectric point of *r*-phycocyan, which according to recent determinations by K. O. Pedersen is situated at P_H 4.85. The sedimentation is independent of P_H within this region and has a mean value of 11.3×10^{-13}, identical with the value previously found for *r*-phycocyan from *Porphyra* at P_H 4.6,[2] *viz.*, 11.1×10^{-13}. On both sides of this region the sedimentation falls off very rapidly with change in P_H and then reaches two new regions where it is again independent of P_H. The one at the alkaline side stretches from P_H 6.8 to 8.0. The sedimentation constant has the mean value 5.9×10^{-13}, identical with the values previously found[1] for *r*-phycocyan from *Ceramium* at P_H 6.8, *viz.*, 5.59×10^{-13}, and the value for *r*-phycocyan from *Porphyra* at P_H 6.8, *viz.*, 5.98×10^{-13}. The region with constant sedimentation at the acid side is very short. It extends only from P_H 1.5 to 1.8. The sedimentation constant has the mean value 5.8×10^{-13}, which is the same as the value in the alkaline region. When the P_H becomes still more alkaline and still more acid, the sedimentation begins again to change rapidly with P_H. At the alkaline side it decreases, at the acid side increases, with P_H.

Two sedimentation equilibrium measurements at P_H 5.0 gave for the molecular weight the mean value 209,000, which is identical with the value previously found[2] for *r*-phycocyan from *Porphyra* at P_H 4.6, *viz.*, 206,000. For phycocyan from *Ceramium* the molecular weight 106,000 has previously been found in the region P_H 6.8 to 7.9.[1]

An equilibrium run carried out on a phycocyan solution in the alkaline transition region, *viz.*, at P_H 6.2, showed a strong drift in the molecular weight values, an indication that *r*-phycocyan at this P_H contains a mixture of molecules of different weight. In order to determine the nature of these molecules, three sedimentation velocity runs were carried out at the same P_H in a very strong centrifugal field. In Fig. 8 are shown the sedimentation pictures from one of these runs and in Fig. 9 the microphotometric curve of exposure No. 8. It is quite evident that the solution contains two different and well-defined molecular species. In Table IV the results of these determinations are given.

TABLE IV

r-PHYCOCYAN, SEDIMENTATION VELOCITY MEASUREMENTS

Centrifugal force about 200,000 times gravity; thickness of column of soln., 1.2 cm.; concentration of protein soln., 0.09%; solvent, 0.080 *M* in KH_2PO_4 and 0.020 *M* in Na_2HPO_4; P_H of soln., 6.2; optical conditions as in Table IV.

Number of run	$S_{20°} \times 10^{13}$ From first boundary	From second boundary
1	5.9	11.5
2	5.5	11.5
3	6.7	11.6

The values of the sedimentation constant derived from the movement of the first boundary are identical with the value found for the half-molecules and the sedimentation constant calculated from the second boundary is the same as that characteristic of the undissociated phycocyan molecules.

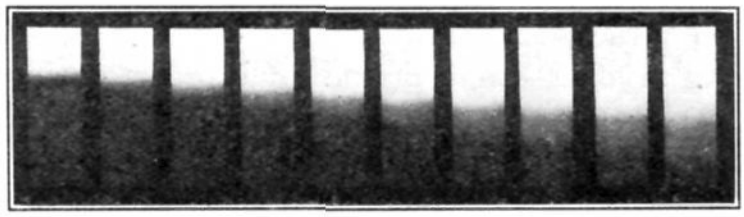

Fig. 8.

In the transition range between the region of non-dissociation and the alkaline region of complete dissociation into half-molecules, the *r*-phycocyan therefore consists of a mixture of these two kinds of molecules.

On the acid side of the region of normal, undissociated molecules the transition range is very narrow and therefore no attempt to analyze the solution in a very strong centrifugal field was made. In the acid region of sedimentation constant 5.8×10^{-13} no equilibrium measurements were performed because of the difficulty of preventing decomposition in the solution during the long time necessary for an equilibrium run. The fact, however, that a sedimentation constant around 5.8×10^{-13} corresponds to a molecular weight half that of normal phycocyan also in the case of serum globulin strongly supports the assumption that *r*-phycocyan is dissociated into half-molecules in the acid region P_H 1.5 to 1.8.

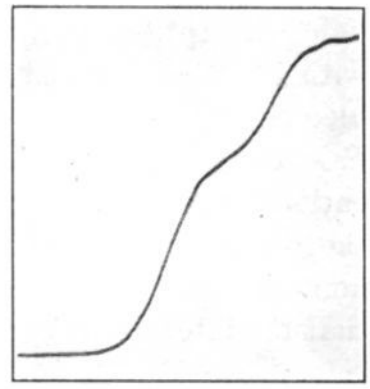

Fig. 9.

The expenses connected with this investigation, including the considerable cost of the construction of the new high-speed ultracentrifuge, have been defrayed by grants from the Nobel Fund of Chemistry, from the Rockefeller Foundation and from the Foundation "Therese och Johan Anderssons Minne."

Summary

1. The sedimentation constant of *c*-phycocyan has been determined in fresh extracts of *Aphanizomenon flos aquæ* and found identical with the value for *c*-phycocyan purified by means of crystallization with ammonium sulfate. *c*-Phycocyan therefore probably exists in the cells of the algae with the same molecular weight, 208,000, as in the purified laboratory product.

2. The sedimentation constant of *r*-phycoerythrin has been determined in fresh extracts of *Ceramium rubrum* and found to be identical with the

value for *r*-phycoerythrin purified with ammonium sulfate. Native *r*-phycoerythrin therefore probably is identical with the purified product with regard to molecular weight.

3. Phycoerythrin from *Polysiphonia urceolata* has been studied by means of the ultracentrifugal methods. The protein was not quite homogeneous but both the sedimentation constant, 12.4×10^{-13}, and the molecular weight, 196,000, were of the same order of magnitude as for normal *r*-phycoerythrin. The P_H stability range was also the same.

4. The sedimentation constant of the phycoerythrin modifications from three Mediterranean algae, *viz.*, *Bornetia secundiflora*, *Griffithsia furcellata* and *Sebdenia monardiana*, has been determined. *Sebdenia* phycoerythrin gave the same value for the sedimentation constant as *Ceramium* phycoerythrin, *viz.*, 11.3×10^{-13}. The constants for *Bornetia* and *Griffithsia* phycoerythrin were slightly higher, *viz.*, 12.1×10^{-13}. It is believed that this deviation is due to inhomogeneity of the solutions studied. The P_H stability range was the same as for normal phycoerythrin. With regard to light absorption, the *Bornetia* phycoerythrin was found to be identical with the *Ceramium* phycoerythrin, while *Griffithsia* phycoerythrin resembled closely the phycoerythrin from *Polysiphonia*. The light absorption of *Sebdenia* phycoerythrin differs considerably from that of the other modifications as already pointed out by Gaetano. A semi-quantitative study of the fluorescence of the phycoerythrin modifications did not reveal any marked difference in the wave length of the fluorescence light but showed that the intensity is considerably lower for the phycoerythrin from *Griffithsia*.

5. The behavior of *r*-phycocyan has been studied carefully within a wide P_H range. From P_H 2.5 to 5.0 the sedimentation constant is 11.3×10^{-13} and the molecular weight 209,000. On both the alkaline and the acid side of this normal region, which includes the isoelectric point (P_H 4.85), is a region where the sedimentation constant is $5.8\text{–}5.9 \times 10^{-13}$ and the molecular weight half the normal value. These two regions are connected to the normal region by transition regions where the *r*-phycocyan solution contains molecules of the normal and of half the normal weight at the same time. In solutions more acid than P_H 1.5 or more alkaline than P_H 8.0, the phycocyan molecule begins to decompose and form systems, inhomogeneous with regard to molecular weight.

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