LXXVI. THE MOLECULAR WEIGHTS OF PHYCOERYTHRIN AND PHYCOCYAN. I

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THE molecular constants of phycoerythrin and phycocyan from various algae have previously been studied in this institute. Svedberg & Lewis [1928] found that at pH 6.8 the phycocyan from *Ceramium rubrum* of the Rhodophyceae family had half the molecular weight of the phycoerythrin from the same alga. Svedberg & Katsurai [1929] studied the phycoerythrin and phycocyan from another of the Rhodophyceae, *Porphyra tenera*, and the phycocyan from a species of the Cyanophyceae, *Aphanizomenon flos aquae*. This work indicated that at the isoelectric points the molecular weights of all three were about the same, but in more alkaline solutions the phycocyan seemed to dissociate into half-molecules. Svedberg & Eriksson [1932] determined the pH -stability diagram of *Ceramium-phycocyan*, and found that its sedimentation constant in the pH region around the isoelectric point was almost identical with that of phycoerythrin. In a more alkaline region it dropped to a value that might correspond to half-molecules. In view of the regularities lately found among the protein molecular weights it was considered of importance to determine accurately the molecular weights of phycoerythrin and phycocyan and learn more about the latter's dissociation.

These investigations were carried out on phycocyan and phycoerythrin from *Ceramium rubrum*. The proteins were prepared by the method described previously [Svedberg & Eriksson, 1932].

The pH-stability diagram and molecular weight of phycoerythrin

The sedimentation constant of phycoerythrin was determined at various pH in the high-speed ultra-centrifuge. The results are summarized in Table I. The protein is stable and homogeneous in the pH region 3–10. The mean value of the sedimentation constant is 12.0.¹ Between 10.5 and 11.4 it is partly dissociated into molecules of lower molecular weight. At 11.4 all the material of normal molecular weight has vanished. The sedimentation constant of the dissociation product is about 2. A slight amount of an intermediate dissociation component with a sedimentation constant of about 5 has also been observed. Below pH 3 the protein aggregates into a high-molecular heterogeneous product.

The determinations in Table I were made immediately after mixing the solutions. The dissociation, however, increases with time, as may be seen from Table II. Before centrifuging the solutions were left for 2–3 days at room temperature.

Molecular weight determinations were made by means of sedimentation equilibrium runs. For the measurement of the concentration gradient the

¹ Sedimentation constants are expressed in units of 10^{-13} and diffusion constants in units of 10^{-7} .

Table I. *Phycoerythrin*

Centrifugal force about 160,000 times gravity, in the last three runs 260,000, protein conc. about 0.1 %, thickness of column of solution 0.3–1.2 cm., source of light mercury lamp, plates Imperial process, metol-hydroquinone developer.

Solvent	pH	Total <i>M</i>	Light filter	s_{20}	
HCl, NaCl	2.17	0.16	NiO	20.1*	Heterogeneous
"	3.27	0.18	"	12.5	
HOAc, NaOAc, NaCl	3.6	0.22	"	12.2	
"	4.6	0.22	Wratten 77	11.6	
HOAc, NaOAc	4.6	0.20	"	12.2	
"	5.3	0.20	NiO	12.1	
KH ₂ PO ₄ , Na ₂ HPO ₄ , NaCl	5.8	0.24	"	11.9	
"	5.9	0.25	"	11.7	
"	6.3	0.22	"	11.8	
"	6.8	0.22	"	11.9	
"	6.8	0.22	Cl ₂ , Br ₂	12.2	
"	6.8	0.28	Wratten 77	12.0	
"	7.2	0.22	NiO	12.4	
"	7.8	0.24	"	11.8	
KH ₂ PO ₄ , Na ₂ B ₄ O ₇ , NaCl	8.3	0.25	"	12.1	Heterogeneous
"	8.6	0.25	"	12.1	
"	9.00	0.20	"	11.6	
Na ₂ CO ₃ , Na ₂ B ₄ O ₇ , NaCl	9.3	0.25	"	12.0	
"	9.84	0.25	"	11.6	
"	10.5	0.25	"	11.6	
Na ₂ HPO ₄ , NaOH, NaCl	11.0	0.25	"	11.2* + 3.29	
"	11.4	0.26	"	1.88	
"	11.7	0.22	Wratten 77	4.98, 2.0	
"	11.9	0.22	"	2.1	
		Mean		12.0	

* Not used for the calculation of mean value.

Table II. *Time effect on phycoerythrin at alkaline pH*

Solvent	pH	Total <i>M</i>	Light filter	s_{20}
Na ₂ B ₄ O ₇ , Na ₂ CO ₃ , NaCl	9.84	0.25	NiO	11.6 + 11.5
"	10.5	0.25	"	2.2
Na ₂ HPO ₄ , NaOH, NaCl	11.0	0.26	"	11.7 + 5.48, 1.58
"	11.3	0.26	"	4.68, 1.75
"	11.9	0.22	Wratten 77	

refractometric scale method was used. This procedure is more accurate than the earlier light absorption method, and allows a better control of the establishment of the equilibrium. Two determinations were made under the following conditions: protein concentration 0.22 %, pH 6.8 (0.01 *M* in KH₂PO₄ and Na₂HPO₄, 1 % NaCl), exposures made after 100–150 hr., speed 83 rev. per sec., temperature 293°, thickness of column of solution 0.4 cm., length 0.5 cm. The values obtained were 286,000 and 298,000, mean 293,000. The constancy of molecular weight demonstrates the homogeneity of the protein.

Tiselius & Gross [1934] obtained the value 4.00 for the diffusion constant of phycoerythrin and this combined with the sedimentation constant 12.0 gives the value 290,000 for the molecular weight.

The pH-stability diagram and molecular weight of phycocyan

Some sedimentation velocity runs were made at different *pH*. They showed good agreement with the stability diagram already published [Svedberg & Eriksson, 1932]. The values given in that paper, however, had not been corrected for the density and viscosity of the solvent. The protein is homogeneous with regard to molecular weight in the *pH* regions 2.5–6 and 7–8.5. The value of the sedimentation constant is 11.4 in the former and 6.2 in the latter region. (These figures include the corrected old as well as the new determinations.) Between *pH* 6 and 7 the protein consists of a mixture of both kinds of molecules. The results are summarized in Table III.

Table III. *Phycocyan*

Centrifugal force 100,000–200,000 times gravity, protein concentration about 0.1 %, thickness of column of solution 0.6–1.2 cm., light-filter nickel oxide glass, other conditions as in Table I.

Solvent	<i>pH</i>	Total <i>M</i>	s_{20}	
			A	B
HCl, KCl	2.0	0.05	10.6*	—
" "	2.5	0.05	11.5	—
" "	3.0	0.04	11.5	—
NaOAc, HOAc, NaCl	3.5	0.22	11.0	—
" " "	3.6	0.24	11.3	—
" " "	3.9	0.24	11.3	—
" " "	4.0	0.22	11.7	—
" " "	4.0	0.24	11.3	—
" " "	4.5	0.28	11.7	—
" " "	5.0	0.22	12.3	—
" " "	5.0	0.22	11.9	—
" " "	5.0	0.22	11.5	—
KH ₂ PO ₄ , Na ₂ HPO ₄	5.5	0.10	11.1	—
KH ₂ PO ₄ , Na ₂ HPO ₄ , NaCl	5.5	0.22	11.6	—
KH ₂ PO ₄ , Na ₂ HPO ₄	5.8	0.10	11.1	—
" "	6.2	0.10	11.5	5.9
" "	6.2	0.10	11.5	5.5
" "	6.2	0.10	11.6	6.7
KH ₂ PO ₄ , Na ₂ HPO ₄ , NaCl	6.5	0.22	10.6*	6.28
" "	6.8	0.22	10.3*	6.06
KH ₂ PO ₄ , Na ₂ HPO ₄	7.1	0.10	—	6.8
KH ₂ PO ₄ , Na ₂ HPO ₄ , NaCl	7.2	0.22	—	6.34
KH ₂ PO ₄ , Na ₂ HPO ₄	7.3	0.10	—	6.4
KH ₂ PO ₄ , Na ₂ HPO ₄ , NaCl	7.5	0.22	—	6.13
KH ₂ PO ₄ , Na ₂ HPO ₄	7.7	0.10	—	6.8
KH ₂ PO ₄ , Na ₂ HPO ₄ , NaCl	7.8	0.22	—	6.15
KH ₂ PO ₄ , Na ₂ HPO ₄	8.0	0.10	—	6.08
KH ₂ PO ₄ , Na ₂ B ₄ O ₇ , NaCl	8.0	0.20	—	6.3
" " "	8.5	0.20	—	6.00
Na ₂ HPO ₄	8.6	0.10	—	5.6*
Na ₂ HPO ₄ , NaOH, NaCl	9.1	0.21	—	4.1*
Mean			11.4	6.24

* Not used for the calculation of mean value.

Molecular weight determinations by means of sedimentation equilibrium measurements were made in both these regions. The data are summarized in Tables IV and V. The molecular weights found are 273,000 for the normal and 146,000 for the dissociated protein.

The diffusion constant for the undissociated phycocyan is 4.05 [Tiselius & Gross, 1934] and for the dissociation product 4.58 [A. G. Polson, unpublished]. Combined with the sedimentation constants these values give the molecular weight 272,000 for the undissociated and 131,000 for the dissociated molecules.

Table IV. *Undissociated phycocyan* $pH=5.0$ (0.02 *M* acetate, 0.2 *M* NaCl), speed 83.0 rev. per sec., cells 1.0 and 1.2 cm.

Exp. no.	Molecular weight
1	263,000
2	284,000
3	264,000
4	281,000
Mean	273,000

Table V. *Dissociated phycocyan* $pH=7.5$ (*M*/15 phosphate, 1 % NaCl); speed 130 rev. per sec.
other conditions as in Table IV

Exp. no.	Molecular weight
1	147,000
2	143,000
3	151,000
4	141,000
Mean	146,000

DISCUSSION

The molecular weights of phycocyan and phycoerythrin have been determined by means of two independent methods, giving within limits of experimental error the same results. The mean values for the molecular weights obtained are, in the pH regions around the isoelectric points, for phycoerythrin 291,000 and for phycocyan 273,000. In these regions, therefore, the proteins are of almost identical molecular weight. The molecular weight of the dissociated phycocyan is 138,000, which is half the normal value. This demonstrates that the phycocyan splits up into half-molecules.

Svedberg has advanced the hypothesis that all protein molecules are simple multiples of a common submolecule of molecular weight 17,600, obtained by dividing all the determined protein molecular weights by simple numbers [Svedberg, 1937]. In this system phycocyan and phycoerythrin belong to the group containing 16 submolecules, the calculated molecular weight for this group being 282,000. This group also contains the plant globulins amandin, excelsin and edestin. The dissociated phycocyan should contain 8 submolecules. No proteins with molecular weights falling between these two groups are known.

The discrepancy between these molecular weight values and those found by Lewis, viz. 208,000 for phycoerythrin and 106,000 for dissociated phycocyan may be due to the short time of his centrifuging. His calculations are made from the concentration distribution obtained after 40–50 hr., which, according to later experience, does not allow the establishment of complete equilibrium.

SUMMARY

1. The sedimentation constant of phycoerythrin has been investigated in the pH range 2–12. It is independent of pH from 3 to 10. Its mean value is 12.0 in this region.

2. The molecular weight of phycoerythrin has been determined. Two sedimentation equilibrium runs gave a mean of 292,000. By combining sedimentation and diffusion the value 290,000 was obtained.

3. The sedimentation constant of phycocyan has been recalculated and some new determinations made. For the undissociated molecule the value 11.4 was obtained and for the dissociated molecule 6.2.

4. The molecular weight of phycocyan has been determined. From sedimentation equilibrium measurements the values 273,000 for normal and 146,000 for dissociated phycocyan were found. A combination of sedimentation and diffusion gave 272,000 and 131,000 respectively.

5. The molecular weight determinations on phycocyan confirm the earlier conclusion that the molecules of this protein split into halves when they dissociate.

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