META- AND PYROPHOSPHATE WITHIN THE ALGAL CELL

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Introduction

Comparatively little attention has been given to the pyro and almost none to the meta form of phosphate in plant and animal studies. Since reporting the occurrence of pyrophosphate in muscle in 1928, LOHMANN (**5, 6, 7, 8, 9, 10, 11, 12**) has conducted a number of experiments on the occurrence and transformation of pyrophosphate in animal tissue. He also made a few similar studies with yeast and plant material. Other work in which the pyro form of phosphate was considered was done by MEYERHOF and LOHMANN (**14**), BOYLAND (**1**), LEVITOV (**4**), EGGLETON and EGGLETON (**2**), EMBDEN, HEFTER, and LEHNARTZ (**3**), NEEDHAM and VAN HEYNINGEN (**15**), and ROCHE (**16**). According to LOHMANN (**7**) and BOYLAND (**1**), about one-fourth of the phosphate in yeast is in the pyro form. LEWITOW (**4**) reported that incubation of brewer's yeast did not alter the pyrophosphate content but that when this was done in the presence of glucose there was a considerable loss in pyrophosphate with a simultaneous decrease in orthophosphate. In most of the work, determinations for pyrophosphate might have included metaphosphate. WEISSFLOGG and MENGDEHL (**19**) found meta- and pyrophosphate in the roots of corn only when they used these forms as the source of phosphate in their culture media. Even in these cases they were unable to detect either meta- or pyrophosphate in the upper parts of the plant and concluded that these were converted into the ortho form before they reached the stems and leaves. Dr. RALPH MORGAN (private conversation) said that he believed metaphosphates were an important form in the living organism because they readily form complexes. An investigation was therefore undertaken to determine whether metaphosphate was present in living green plants and, if so, its relation to the presence of the ortho and pyro forms.

Experimentation

The ease with which meta- and pyrophosphates are converted to the ortho form makes it desirable to use, if possible, the intact living organism. The senior author and colleagues (**17, 18**) have shown that the ALLISON magneto-optic apparatus permits the direct detection of compounds within sufficiently small and transparent plants. The only requirement is that sufficient light be transmitted through the organism to give the minimum or minima of the compound being studied.

A pure culture of *Chlorella pyrenoidosa*[1] was used in most of the studies but similar results were obtained with other algae.

[1] The culture was obtained from Dr. D. APPLEMAN of the University of California at Los Angeles and was of a strain isolated by Dr. R. EMERSON of the California Institute of Technology.

Preliminary trials were made to determine if metaphosphate was present within the algal cell. A suspension of algae in a nutrient solution in which all of the necessary ions were present was placed in an observation tube and the minima for KPO_3 were sought. These were found to be present and angle readings[2] to within $\pm 0.5°$ were made. The suspensions were filtered through hardened filter paper, and readings were made on the filtrate. The filtrate usually showed no metaphosphate; and when the minima for this compound could be seen, the angle readings were very small.

After the preliminary tests showed the presence of metaphosphate within the algal cell, a study was made of its formation from the orthophosphate in the solution.

A culture solution was made up as follows:

$MgSO_4$	10	p.p.m.
$CaSO_4$	10	p.p.m.
KCl	12.5	p.p.m.
KNO_3	10	p.p.m. to 100 p.p.m.

A small amount of a heavy algal suspension from a culture in which all or nearly all of the phosphate had been utilized was added to this solution. Potassium acid phosphate was added to the suspension just before making the readings. Readings for K_3PO_4[3] and KPO_3 were made immediately after

[2] Angle readings vary directly with the concentration. BISHOP, E. R., DOLLINS, C. B., and OTTO, I. G. Magneto-optic rotation method for quantitative determination of calcium. Jour. Amer. Chem. Soc. 55: 4365–4370. 1933.

The data presented in table II were obtained by the senior author during the absence of the junior author. The readings, although much higher than those in table I, which the junior author observed, represent lower concentrations. Readings obtained by different observers may vary greatly for a given concentration as is shown in the following data.

COMPARISON OF ANGLE READINGS FOR THE SAME CONCENTRATION BY TWO OBSERVERS

OBSERVER	ANGLE READINGS IN DEGREES ($\pm 0.5°$) FOR CONCENTRATIONS OF						
	1×10^{-11}	1×10^{-10}	1×10^{-9}	1×10^{-8}	1×10^{-7}	1×10^{-6}	1×10^{-5}
Junior author	6.0	13.0	17.5	19.5	22.0	24.0	25.5
Senior author	18.5	34.5	46.5	65.5*	.....	.....	.....

* Readings above 60° are of very doubtful value.

The readings of the junior author are considerably lower and those of the senior author considerably higher than those obtained by most observers for similar concentrations. Readings for the same concentrations are usually quite constant for an individual over a considerable period of time but may vary over longer periods. Thus the readings of the junior author decreased gradually during a period of two years while those of the senior author increased greatly after eye strain from too constant observing. The data in table I were obtained at a time when a reading of 39° by the junior author corresponded to a concentration of about 1×10^{-4}.

[3] Readings for K_3PO_4 were made because it has been impossible to find minima for

preparing the suspension and again after the algae had been in contact with the solution for an hour or more. A final reading was usually made a day or two later.

It may be seen in table I that part of the orthophosphate was converted into the meta form. The final readings for the metaphosphate were usually higher than those for the orthophosphate when the relative amounts of algae

TABLE I

TRANSFORMATION OF ORTHO- TO METAPHOSPHATE WITHIN THE ALGAL CELL
(Angle readings vary directly with the concentration)

TEST NUMBER	ANGLE READINGS (± 0.5) IN DEGREES*					
	INITIAL		AFTER 1 TO 3 HOURS		AFTER 1 TO 2 DAYS	
	K_3PO_4	KPO_3	K_3PO_4	KPO_3	K_3PO_4	KPO_3
1	28.5	9.5	15.0	21.0	14.5	15.5
2	37.0	9.5	28.5	14.5	16.0	18.5
3	27.0	8.0	17.0	16.5	.....	.....
4	22.0	8.0	7.5	14.5	6.0	8.0
5	22.0	7.5	12.0	13.0	9.0	16.5
6	20.0	9.0	12.5	18.0	5.5	16.5
7	31.5	9.0	29.5	12.5	26.5	12.0
8	36.5	12.5	33.0	13.5	26.0	9.5
9	28.5	12.0	23.5	13.0	23.0	9.0

* Observations by junior author. See footnote page 200.

and phosphate were such that the phosphate was rapidly disappearing. When, however, the amount of phosphate as compared with the algae was greater, the amount of metaphosphate appeared to hold a fairly constant level and the orthophosphate disappeared more slowly.

A second study was made to determine if pyrophosphate appeared as an intermediate form between the ortho- and metaphosphates. Algae used for this work had been growing for a long period without the addition of phosphate to the culture solutions. Suspensions were made in solutions of the composition previously stated. Examinations of the suspensions showed no ortho- or pyrophosphate. Metaphosphate was present in the suspensions made up on the first two days but it could not be detected in those made up later. Three aliquots of the suspension, two with algae and one from which the algae had been filtered were used each time. Orthophosphate was added to one of the unfiltered aliquots and to the filtered aliquot. Examinations were made before and just after the addition of the phosphate, and again after an hour or more. Final readings were usually made the following day.

salts of HPO_4^{--} or $H_2PO_4^-$. When such salts are dissolved, minima are obtained for the salt of PO_4^{---} (the amount of phosphate determined being equivalent to the amount of the particular cation present unless this cation had been added in excess of the phosphate), the hydroxide of the cation, and H_3PO_4. In this work an excess of the cation of the salt investigated was always present.

TABLE II

RELATIONSHIP OF ORTHO-, PYRO-, AND METAPHOSPHATES WITHIN THE ALGAL CELL
(ANGLE READINGS VARY DIRECTLY WITH THE CONCENTRATION)

TEST NUMBER	TYPE OF SOLUTION	ANGLE (± 0.5) READINGS IN DEGREES*								
		INITIAL			AFTER 1 HOUR			AFTER 2 OR MORE HOURS		
		PO_4^{---}	$P_2O_7^{----}$	PO_3^-	PO_4^{---}	$P_2O_7^{----}$	PO_3^-	PO_4^{---}	$P_2O_7^{----}$	PO_3^-
1	Without PO_4	0-†	0-	43.5	0-	0-	43.5	.....	.....	.....
	With PO_4	44+	0-	43.5	+‡	14.5	46.0	.....	.....	.....
	With PO_4 (filtered)	44+	0-	0-	+‡	0-	0-	.....	.....	.....
								After 2 hours		
2	Without PO_4	0-	0.5§	19.5	0-	0-	19.5	0–	0–	19.5
	With PO_4	59.5	0-	19.5	57.5	26.0	24.0	56.5	27.0	29.5
	With PO_4 (filtered)	59.5	0-	0-	59.5	0-	0-	59.5	0–	0–
								After 24 hours		
3	Without PO_4	0-	0-	0-	0-	0-	0-	0–	0–	0–
	With PO_4	60.5	0-	0-	59.5	11.0	10.0	55.0	25.5	44.5
	With PO_4 (filtered)	60.5	0-	0-	60.5	0-	0-	60.0	0–	0–
4	Without PO_4	0-	0-	0-	0-	0-	0-	0–	0–	0–
	With PO_4	60+	0-	0-	60+	20.0	20.0	60+	46.0	50.0
	With PO_4 (filtered)	60+	0-	0-	60+	0-	0-	60+	0–	0–

* Observations by senior author. See footnote page 200.
† 0-, minima not seen at 0°.
‡ With PO_4 present but angle reading not determined.
§ Probably contamination in tube.

Pyrophosphate soon appeared after the addition of orthophosphate to the suspensions and persisted over the period in which the determinations were made. The amount present shortly after the addition of the orthophosphate was sometimes greater than that of the metaphosphate, but later determinations always showed an amount of metaphosphate greater than that of the pyrophosphate. The results of these tests are shown in table II.

Discussion

The transformation of orthophosphate to the meta form and the persistence of metaphosphate after the ortho and pyro forms could no longer be detected indicates that the meta form is important in the metabolic processes of algae. Some of the phosphate in biological material determined by certain investigators as pyrophosphate may have originated from the meta form since their methods did not distinguish between the two. According to WEISSFLOG and MENGDEHL (**19**), meta- and pyrophosphates are rapidly transformed into the ortho form after they are absorbed by corn plants. The fact that they found no meta- or pyrophosphate in the stems and leaves may have been because the amounts were too small to be determined by their method. MENGDEHL (**13**) was able to determine meta- and pyrophosphate which he added to plant material. It seems, therefore, unlikely that if considerable amounts were present in the material from corn plants used by WEISSFLOG and MENGDEHL (**19**) they would have been converted to the ortho form by the time the determinations were made. The results in table II show an amount of pyrophosphate greater than that of the meta form only for a time after the orthophosphate had been added to cultures deficient in phosphate. Algae grown for several months with adequate phosphate and then suspended in a solution of the composition given above also showed metaphosphate to be present in greater amounts than the pyrophosphate. The circle readings were as follows: $Mg_3(PO_4)_2$, 50°[4]; $Mg_2P_2O_7$, 26.5°; and $Mg(PO_3)_2$, 41.5°. Examinations of the solution before the addition of the algae showed no meta-, ortho-, or pyrophosphate. These results indicate that pyrophosphate might be present only as an intermediate step between the ortho and meta forms.

Summary

Living algal cells were examined by means of the ALLISON magneto-optic apparatus for the presence of meta- and pyrophosphates. These were found to be present except where the algae had been in phosphate-deficient media for a long time. Metaphosphate was present after the ortho and pyro forms could no longer be detected. The transformation of ortho- to metaphosphate

[4] Readings not made above 51°.

and the persistence of the metaphosphate indicates that this form is important in the metabolic processes of algae.

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