X-RAY DIFFRACTION OF ALGAL STARCHES

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SUMMARY

The starches of a number of green and red algae were examined by X-ray diffraction. Some showed the X-ray spectrum characteristic of cereal starches, others that characteristic of tuber starches. The former type was not observed in red algae.

In many cases in which neither of these spectra was obtained the diagrams generally showed only one, more or less well defined line, always at 4.51 Å; they probably represent a new type of starch X-ray spectrum.

In a few cases, only a diffuse ring in the 4–6-Å region could be obtained, although a true starch was present.

In some cases, spectra intermediate between the new type and that of the tuber starches were observed.

The observations have been discussed.

INTRODUCTION

As a continuation of our earlier examination of the starches of some *Odonthalia* species and of an *Ulva* species[1] the starches of a wider series of algae were examined by X-ray diffraction. The main purpose was to extend the data on the nature of algal starches, in particular with a view to the question of their relationship to the starches of higher plants.

The X-ray diagrams of *Odonthalia* starches indicate a definite relationship of these to the tuber starches[1], whereas *Ulva* starch yields a diagram which does not correspond to any type known from starches of higher plants, although, on account of the X-ray diagram of its alcohol-precipitated form, it must be considered a true starch[1].

The question whether algal starches may also show the type of X-ray diagram given by cereal starches and whether the starch modification observed in *Ulva* is normal in algal starches had not been answered so far.

In the course of time we have collected some data bearing on this problem, and we have included the starches of a moss and a fern. The results are reported in the present paper.

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METHODS

Isolation of native algal starches

Since the present investigation concerns a total of about 30 red and green algae varying widely in chemical make-up and availability as well as in the properties of their starch grains, only the principles which guided the isolation of the latter can be indicated here. The marine species were collected in the Puget Sound region.

As soon after collection as possible, the algae were introduced into a fairly concentrated I–KI solution in order to stain their starch grains, to increase the latter's weight, and to stop possible amylolytic reactions. The material was then thoroughly ground, in a Waring blendor when large quantities of starch-rich material were present (*Rhodymenia pertusa, Constantinea subulifera*), in a mortar when only a little was available. After the grinding, it was often advantageous to add much water to facilitate the next two steps: straining through cloth (or plankton-gauze) and fractionated sedimentation. In starch-rich material, the latter process was effected either in tall, cylindrical vessels which were allowed to stand undisturbed for some time, or in small tubes which were spun in a centrifuge. Many repetitions were as a rule necessary to render the material reasonably free from impurities. In the more difficult cases, shaking of the starch suspensions with butanol in a separatory funnel was often found very helpful; proteins and pigments have a tendency to accumulate in the upper (butanol) layer, whereas a relatively pure starch collects in the lower, aqeuous phase. This method has previously been employed successfully in the isolation of paramylum from *Euglena* cells[2]. Finally, in those cases where only very limited quantities of suspension strongly contaminated with viscous matter were available, a certain enrichment of the starch could be achieved by the use of a "robot" consisting of 2 flat, rectangular, vertical glass plates covering each other completely but held apart at the top end by a thin coverslip. The starch suspension was introduced at this end; flowing downwards through the narrow passage between the plates, it left many of the contaminating sticky particles behind.

The purity of some of the obtained samples was checked with the anthrone method[3] and also by converting the starch to glucose with an enzyme mixture from *Cryptochiton*[4]; in the latter case, the glucose was subsequently assayed manometrically with the aid of notatin[5]. Some of the red algae (*Constantinea*!) yielded preparations of well-nigh 100% purity. However, the present authors wish to stress that most of their samples were still far from this point.

X-ray examination

The X-ray diagrams were obtained on flat film at a distance of 40 mm from specimens 0.5 mm thick. Ni-filtered Cu K_α radiation was used (30–35 kV/100 mA) collimated by a glass capillary 0.5 mm wide and 40 mm long.

Since dryness may diminish the crystallinity of starch, most samples were examined both in the air-dry condition and after being stored in a container with some moist filter paper until immediately before the irradiation.

According to the literature and our own experience with potato starch, the cereal and tuber types of starch X-ray spectra are not affected when the starch is coloured blue by iodine. However, the experience with a few of the heavily stained samples of our algal starches was that the X-ray diagrams did not show any diffraction in the

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Figs. 1–4. Quadrants of X-ray powder diagrams of starches from:

Fig. 1. a. *Polypodium vulgare*; b. *Hydrodictyon reticulatum*; c. *Rhizoclonium* sp.; d. *Enteromorpha intestinalis*.

Fig. 2. a. Potato; b. *Chara* sp.; c. *Volvox aureus*; d. *Codium fragile*.

Fig. 3A. a. *Laurencia spectabilis*; b. *Spongomorpha coalita*; c. *Rhodymenia pertusa*; d. *Ptilota pectinata*.

Fig. 3B. a. *Spirogyra sp.* 1; b. *Dunaliella viridis*; c. *Mnium affine*; d. *Codium Setchellii*.

Fig. 4. a. *Spirogyra* sp. 2; b. *Farlowia mollis*; c. *Enteromorpha* (with high iodine content).

○ marks 4.45-Å ring in Fig. 1.
● marks 4.51-Å ring in Figs. 2 and 3.
× marks 5.17-Å rings in Figs. 2 and 3.

angular region in which starch reflections will appear. Only a diffuse ring appeared close to the centre of the diagram, which probably must be due to "white" radiation diffracted by the iodine (Fig. 4c). Apparently the longer, characteristic Cu K_α wavelength is too much absorbed by the high concentration of heavy iodine atoms. With decreasing intensity of the iodine staining, the central ring disappears and the intensity of the starch diffraction improves.

It therefore appeared advisable in the staining procedure applied during the starch isolation to use the smallest possible quantity of iodine and to remove this substance later by allowing it to evaporate, or by treating the sample with a solution of hypo-sulphite prior to X-ray examination.

The present data result from the examination of 66 X-ray diagrams covering 42 samples of starch from 30 species. In 4 of these species, however, the results had to be discarded on the basis of obvious low starch content of the samples.

RESULTS

The starches examined are listed in Table I according to the types of their diagrams. Four main types could be distinguished:

1. A type showing the spectrum of the cereal starches, known as the starch A-spectrum (Fig. 1). This spectrum was not obtained from starches of red algae.

2. A type showing the spectrum of the tuber starches, known as the starch B-spectrum (Fig. 2), which was observed in both red and green algae.

3. A type showing only one, more or less intense and slightly broadened diffraction line at 4.51 Å, as observed earlier in the spectrum of *Ulva* starch[1] (Fig. 3). This may be called the U-spectrum. Generally there is a rather intense diffuse scattering around the centre of the diagram and a diffuse ring, forming a background to the 4.51 Å ring and extending mainly on the outside of the latter (Fig. 3A). In a number of diagrams these diffuse rings are less conspicuous and then the 4.51 Å ring is more intense (Fig. 3B). In most of these diagrams, there are sharp rings (usually of slight intensity) which are due to mineral contaminations. The most conspicuous one of these rings corresponds in diameter to the most intense ring of α-quartz and may be caused by particles from the mortar; small glass-like splinters frequently occurred in the samples. The rings outside the 4.51-Å ring in Fig. 3Ba (*Spirogyra*) correspond to $BaSO_4$ reflections, which can be explained from the fact that *Spirogyra* sometimes contains this substance[6].

4. A type showing only diffuse rings, which may be subdivided into: Type 4a, showing a rather heavy diffuse ring in the 4–6-Å region (Fig. 4a) and Type 4b, showing

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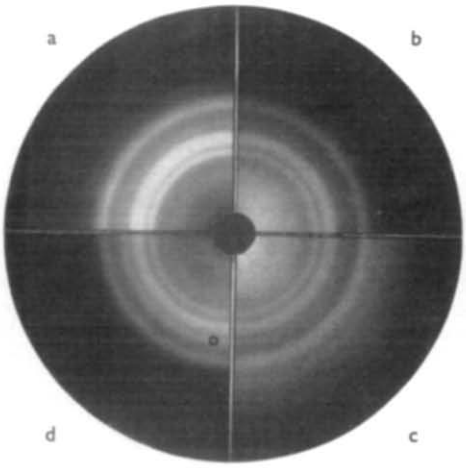

Fig. 1.

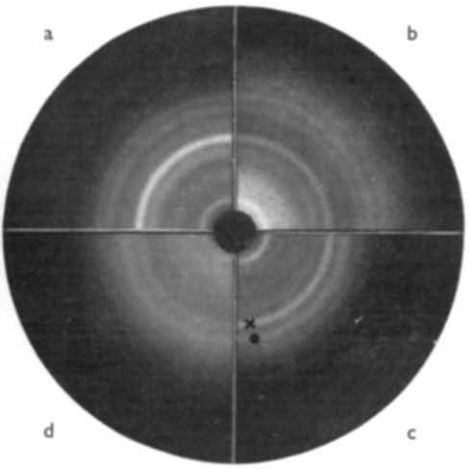

Fig. 2.

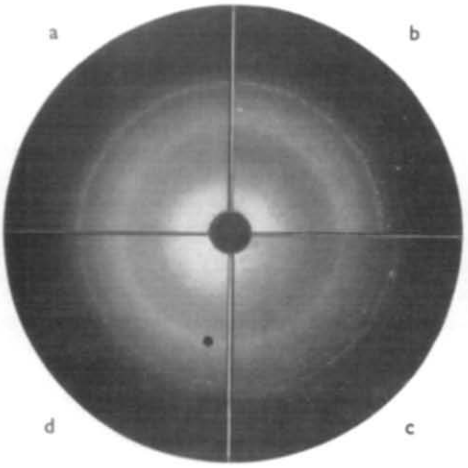

Fig. 3A.

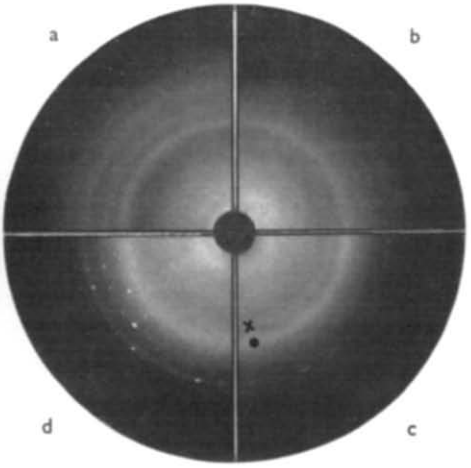

Fig. 3B.

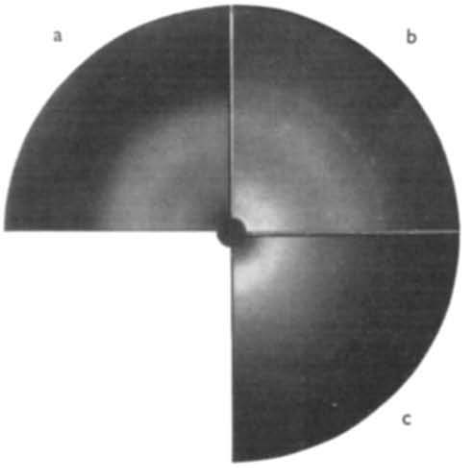

Fig. 4.

a diffuse ring in the 4–5-Å region and a diffuse blackening in the centre, which is different from that due to the iodine content of the starch in that it reaches the central spot (Fig. 4b).

In Table I we have also mentioned the grain size of the starches and the reaction with iodine as observed under the microscope when air-dry samples were placed in a drop of I–KI solution. In many samples no definite grains could be distinguished because of clotting or coalescing into a homogeneous mass.

TABLE I

SURVEY OF STARCHES EXAMINED AND THEIR X-RAY SPECTRA

Algae	*Spectrum*	*Grain size in μ*	*Iodine reaction*
Hydrodictyon reticulatum	A	2–5	+
Rhizoclonium sp.	A	4–7	+
Enteromorpha intestinalis	A; 4.45-Å ring intensified	2–3	+
Haematococcus pluvialis	A; very weak	—	+
Chara sp.	B	—	+
Volvox aureus	B; 4.51-Å ring intensified	2–3	+
Codium fragile	B; 4.51-Å ring intensified	2–3	+
*Constantinea subulifera**	B	—	+
*Plocamium pacificum**	B	6–9	+
Spirogyra sp. 1	U (3B)	—	+
Spongomorpha coalita	U (3A)	—	+
Cladophora sp.	U (3A)	5–12	+
Codium Setchellii	U (3B)	2–3	+
Dunaliella viridis	U; (3B) + 5.17-Å line	1–2	+
Unknown fresh water alga	U (3B)	—	+
*Ptilota pectinata**	U (3A)	—	+
*Laurencia spectabilis**	U (3A)	—	+?
*Rhodymenia pertusa**	U (3A)	—	+
*Rhodymenia palmata**	U (3A)	—	+
*Ahnfeltia gigartinoides**	U (3A)	—	+
Spirogyra sp. 2	diffuse } type 4a	4–5	+
Nitella sp.	diffuse } type 4a	—	+
*Erythrophyllum delesseroides**	diffuse } type 4b	—	—
*Farlowia mollis**	diffuse } type 4b	—	—
Polypodium vulgare (fern)	A	5–10	+
Mnium affine (moss)	U; (3B) + 5.17-Å line	3–6	+

* Red alga.
(3A), (3B): Indication whether U-type of Fig. 3A or 3B was observed.

The iodine reaction was regarded as positive when a blue, violet, reddish or brown-violet colour was observed in the greater part of the sample.

Some further notes may be made concerning the diagrams:

1. A 4.51 Å ring, as observed in the diagrams of type 3, is also present in normal B-starch spectra; *e.g.*, of potato starch (Fig. 2a), where it is comparatively faint. In 2 of our algal starches, however, it appears much more intense than in the normal B-spectrum, *cf.* Figs. 2c and d (*Volvox* and *Codium*). Similarly, the normal A-starch spectrum has a very faint 4.45-Å ring which in one of the algal starches (*Enteromorpha*,

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Fig. 1d) appears with much higher intensity than in the normal A-spectrum, represented in Fig. 1a by that of *Polypodium* starch.

2. Two of the starches of type 3 (*Mnium*, Fig. 3Bc, and *Dunaliella*, Fig. 3Bb) show an additional ring corresponding to the strongest B-starch ring (5.17 Å), and in fact there is little difference between these diagrams and that of *Codium* starch (Fig. 2d), where the B-spectrum lines are very faint and the 4.51-Å line dominates in intensity.

3. Generally there is a difference between the spectra obtained from the air-dry and those from the moist starches. In some cases (*Plocamium, Chara, Constantinea*) the dry samples produced a diagram of type 4a, whereas the moist samples showed lines of the B-spectrum. Other diagrams of type 4a (*Nitella* and *Spirogyra* sp. 1) became more diffuse after the sample had been moistened, and so did the diagrams of type 4b. Also, in the starches with U-spectra the diagrams of moist starches showed less detail than those of the dry ones. In those cases where both a wet and a dry sample were examined, the classification in Table I has been made according to the diagram showing the most detail. The type-indication is underlined if it refers to a diagram from a moist sample.

4. In all our samples with a B-spectrum, including some very pure samples like those from *Constantinea* and *Plocamium*, the lines of the diagram are considerably less intense than in potato starch. The crystallinity in these starches is therefore relatively low, as was also observed in the *Odonthalia* starches investigated earlier[1].

DISCUSSION

The phenomena mentioned above under 1 and 2 indicate that at least the U- and B-modification may occur together in one sample. In this connection it is also of interest that a sample of starch from *Codium fragile* that had been treated with alcohol during the isolation yielded a U-spectrum, while a weak B-spectrum appeared along with the U-spectrum line when only water had been used. The observations therefore, indicate a definite relationship between the starches producing U- and B-spectra, as did those on alcohol-precipitated *Ulva* and potato starch[1]:

At present it is impossible to say what exactly this relationship might mean in terms of molecular arrangement because insufficient details are known of the crystalline packing of native starches. Though certain unit cell dimensions have been found fitting the spacings observed in the B-spectrum very satisfactorily and suggestive evidence exists for a certain spiral model of the molecules[7], the arangement of the latter in the unit cell is a matter of doubt[7–8].

The general conclusion seems to be justified that in the starches with U-spectrum we are dealing with a type of starch which is a true starch but differs in some way from the types of starch found in higher plants, *e.g.* by its degree of ramification, by incrustations, or by the presence of particular organic or inorganic side groups or the amounts of such groups. These differences apparently prevent crystallization in the forms observed in higher plants and only permit a low degree of molecular order producing but one, more or less distinct X-ray interference.

Variations of this type occur which show more resemblance to the starches of higher plants, so that part of their molecules show a weak tendency to combine into crystallites of the type observed in higher plants, in particular into the B-modification. Then X-ray diagrams will be obtained showing features of both the U- and B-spectrum.

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The correspondence with the higher plant starches may go still further, so that only the well known A- or B-spectrum is produced.

On the other hand, there probably exist variations in which the highest degree of molecular order attainable is still lower than that in the U-type so that only a very diffuse ring is produced in the X-ray diagram. Among these starches, those producing diagrams of type 4a are no doubt true starches; they give a bright blue colour with iodine and their type of diagram was also obtained from starches which, in sufficiently moist condition, could yield a B-spectrum.

As for the samples producing diagrams of type 4b, it is doubtful whether they contain any starch, because only a yellow colour appears with iodine. This doubt is supported by the fact that in one case (*Plocamium*) such a diagram was obtained from a sample which stained yellow with iodine, while a sample collected later and staining violet produced a type 4a diagram in the dry condition and one of type B after moistening. If the first sample were also starch, this would mean that *Plocamium* produces two types of starch, which appears unlikely.

Whatever the material yielding the type 4b diagrams may be, it is probably also present, besides true starch, in a number of samples producing U-spectra, where it might give rise to the diffuse ring appearing as a background of the 4.51-Å line and to the strong diffuse blackening in the centre of the diagram as shown in the spectra of Fig. 3A.

The present X-ray data yield no evidence for the occurrence in the algae investigated of "starches" with a molecular structure of the main chains that is essentially different from that of true starch in that the main chains would not consist of α-1,4-linked glucose residues like, for example, in the paramylon of Euglenaceae[2]. However, the presence of chains with a different structure cannot be excluded by the present data because they yield little information on the amorphous fraction of the samples.

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