

The Influence of Water Content on the Action of Ionizing Radiation on Starch

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In order to elucidate the "protective effect" of water in various biologic materials irradiations of polysaccharides of various water contents have been carried out and some chemical changes measured. It was found that the number of breaks produced in the polysaccharide chains of the molecules per energy absorbed decreases with increasing water content, in quantitative parallelism to the effects in biologic materials. This effect can be explained by assuming that in materials of low water content excitations lead to chemical changes with higher efficiency than in materials containing more water. There were also found chemical effects showing an entirely different dependence on water content.

When studying the induction of mutations by irradiating plant seeds, it was found at an early stage of the investigations that the material showed a radiation sensitivity varying irregularly between the individuals in a sample as well as between different samples. The variation was observed to be greater with sparsely ionizing radiations (such as γ - and X-rays) than with densely ionizing radiations (secondaries produced by neutrons). An analysis of possible causes led to the conclusion that the seemingly irregular variation obtained in experiments with sparsely ionizing radiation could be explained by the influence of the water content of the seeds on the radiation sensitivity.¹

Desiccated seed material with a water content of 6—8 % was found to be most sensitive to X- and γ -rays, and a maximum "protective effect" was observed at a water content of about 20 % (the water content corresponding to minimum sensitivity somewhat depending on the material and on the test reaction studied). The dose reduction factors brought about by increased water content range from 2.5 for mutation rate in barley² to 3—4.5 for inhibition of growth in the same material¹. In contradistinction no dependence of sensitivity on water content has been found using fast neutron irradiation¹⁻⁴. At water contents above 20 % the sensitivity increases for all kinds of radiation, due, partly at least, to an increase in metabolic activities, involving, *e.g.*, chromosome multiplication and initiation of germinative processes⁶.

Qualitatively, the influence of the water content is the same for all radiation effects studied: gene mutation, chromosome aberrations, sterility, growth inhibition and lethality, all these effects possibly resulting from disturbances in the nuclear material. The phenomenon is, decidedly, a very general one, since it has been observed in seeds of many different species (barley ¹⁻⁵, tomato ⁷, pea ⁸, and others) and also in pollen ⁹ and in bacteria ¹⁰.

A more detailed analysis of the ways by which water brings about this very pronounced effect might well furnish important clues as to the mechanisms of action of radiations in general. No physiological or biochemical factors have thus far been found that depend on water content in such a way as to explain the modifying action of water on radiosensitivity ⁶. On the other hand the water content exerts a qualitatively and quantitatively very similar influence on the radiation induced chemical changes in non-living hydrophilic colloids ^{6,11}. Consequently, it seems quite reasonable to look for an explanation on the physicochemical level.

The present paper describes investigations into the radiation induced changes in systems of polysaccharides of the starch type (α -polyglucosides), irradiated at different water contents. In a way these experiments are exploratory, as they strive to find which chemical changes depend on water content in the same manner as the biological effects, and which do not.

EXPERIMENTAL

Samples of potato starch, amylose from potato starch, or maize amylopectin ("Amioca" from National Starch Products) were treated, in desiccators, with streaming air of different relative humidities in the range of 0 to 95 %. When, after 4-6 days at 20°C, equilibrium was reached, the samples were irradiated in glass stoppered test tubes.

γ -Irradiation was carried out at distances 3-18 cm from a 70 Curie ⁶⁰Co source. The (average) dose rates at these distances and in the tubes used were determined actinometrically ¹² by measuring the oxidation of FeSO₄ in 0.8 N H₂SO₄, a G-value of 15.5 Fe³⁺ per 100 eV absorbed being used. At a distance of 3 cm the dose rate amounted to 10⁵ r/h.

Roentgen irradiation was given by means of a therapy apparatus run at 175 kV; filtering 2 mm Al; dose rate 800 r/min. The dose was measured during the irradiation by means of a wave-length independent thimble chamber connected to a valve electrometer and integrating counter calibrated in Roentgens (r).

The analytical methods are mentioned in connection with the experimental results.

RESULTS

Common potato starch becomes soluble in water after irradiation by about 10⁵ r of γ - or X-rays, and aqueous solutions can therefore be investigated above this dose.

Solutions of irradiated starch are acidic. In order to get amounts of acid groups, easy to titrate, starch was irradiated with 10 and 24 Mr and the groups were titrated electrometrically with 10⁻³ M NaOH. Within this dose range, the amount of acid groups is proportional to the dose. Probably a mixture of different groups is obtained, with an average pK_s amounting to 4 or a somewhat lower value. The titrations are disturbed by the presence of starch, and therefore the values obtained somewhat approximate. Up to 16 % water the

Table 1. Effects of γ -radiation in starch.

Water content %	Acid groups	Reducing groups	Dose giving $\eta_{sp} = 2.5$ Mr
	per ion pair (32.5 eV of energy absorbed)		
6.4	0.78	2.0	0.35
13.2	0.54	1.9	0.48
16.0	0.50	2.0	0.60
20.8	1.4	1.8	0.78
Dose range:	10—24 Mr		0.3—1 Mr

yield of acid groups per ion pair* is fairly constant or slowly decreasing (Table 1), whereas a much higher value is obtained at 20 % water. Considering the fact that the decomposition of starch decreases monotonically with increasing water content, as measured by the viscosity (Table 1, last column), there is no simple correlation of acidity to decomposition. The number of reducing groups determined according to Somogyi¹³ (column 3), is totally independent of the water content, and cannot be related at all to the net degradation. The reducing groups are also formed in proportion to the dose. — When intact seeds were irradiated by 5×10^5 r of X-rays the number of glucosidic bonds split was somewhat greater at 17 % H₂O than at 8 % H₂O¹⁴. Obviously biochemical and physiological factors have to be considered in the irradiation of tissue.

It seems logical to assume that the formation of one reducing group, *i.e.*, the hydrolysis of a glucoside bond, leads to a break of the polysaccharide chain (although the formation of a new bond not affecting the reducing group is, of course, not excluded). Since the net degradation of starch, in contradistinction to the formation of reducing groups, depends on the water content at irradiation, it was of interest to estimate the total number of breaks in the polysaccharide chains obtained per unit dose. Obviously chemical reactions other than the hydrolysis of glucoside bonds are responsible for part, at least, of the net degradation.

A prerequisite for a direct determination of the number of breaks in the polysaccharide chains would be, of course, the use of a material permitting the study of aqueous solutions of the unirradiated material. Starch derivatives made soluble by chemical treatments, *e.g.*, the carboxymethyl ether used in preliminary experiments, were regarded unsuitable for the purpose due to a possible disturbing influence of the introduced groups or changes. Moreover, since the average molecular weights of α -polyglucosides obtained, for instance, from determinations of viscosity, are rather uncertain, we preferred the following indirect method of estimating the number of chain breaks at a given degree of degradation.

* The term "ion pair produced" is used for convenience and in a strictly formal way, implying no other meaning than the absorption of 32.5 eV of radiation energy in the medium.

In amylose, irradiated at different water contents by 30 Mr of γ -radiation, the degradation was estimated from the displacement towards shorter wavelengths of the absorption maximum of the complex with iodine, and from the decrease of the optical density at 650 $m\mu$. The number of breaks in the polysaccharide chains was calculated from the reduction values measured at the same optical changes caused by very dilute solutions of α -amylase. Since amylose consists mainly of long unbranched chains, both agents can be supposed to attack the molecules at randomly situated points. The optical properties can be supposed to depend exclusively on the chain length, irrespective of the chemical nature of the end groups. This assumption is supported by the fact that the displacement of the absorption maximum agrees fairly well with that expected from the degree of decomposition calculated (see Table 2), *i.e.*, the absorption curve changes equally after irradiation and enzymatic hydrolysis. The yield of chain breaks per ion pair produced is found to decrease from 4–5 at 6 % H_2O to about 2 at 20 % H_2O .

A similar result was obtained with the branched polysaccharide, amylopectin, from which stable 1 % solutions could be prepared. A 50 % decrease of the viscosity of 1 % solutions was obtained by irradiating desiccated samples

Table 2. Amylose, irradiated with 30 Mr (5.0×10^{19} ion pairs) γ -radiation, at different water contents. Number of chain breaks calculated from the extinction decrease at 650 $m\mu$. The colorimetric determinations were done with 0.0002–0.0006 % amylose solutions containing excess iodine (0.004 %)

Water content %	Decrease of extinction at 650 $m\mu$, %	Maximum extinction at $m\mu$	No. of chain breaks		Maximum extinction expected from degree of hydrolysis, $m\mu$
			per g amylose	per ion pair (32.5 eV of energy absorbed)	
6.6	70	570	$23.5 \cdot 10^{19}$	4.4 (± 2)	555
10.1	49	590	$16.7 \cdot 10^{19}$	3.2 (± 2)	580
13.6	37.5	600	$12.5 \cdot 10^{19}$	2.4 (± 1)	595
19.8	29.0	595	$9.8 \cdot 10^{19}$	1.8 (± 1)	605

Table 3. Doses of γ - and X-rays causing 50 % reduction of the viscosity of 1 % amylopectin solutions, and ionic yields for chain breaks.

Water content at irradiation %	50 % dose of γ -rays, Mr	50 % dose of X-rays, Mr	No. of chain breaks per ion pair (32.5 eV of energy absorbed)
4–6	0.2	0.200	4.7 ± 0.6
14	0.6		1.6 *
20	1.0		0.9 *

* Calculated from dose reduction according to column 2.

(4–6 % H_2O) at 200 kr of X-rays or γ -rays (Table 3). The dose reduction factor for obtaining the same viscosity at higher water contents (about 20 % H_2O) was somewhat greater for this compound than for starch and amylose, amounting to about 5 times. From simultaneous determinations of viscosity and reduction value, the number of breaks at different viscosities could be estimated. Since the reduction value is too low to be measured at 50 % viscosity, an extrapolation backwards had to be done on the reduction/time curve; this extrapolation is justified, the curve being linear at the beginning. The yield of chain breaks per ion pair was found, in agreement with the amylose value, to be about 5 in the desiccated material, and about 1 at 20 % H_2O .

DISCUSSION

Genetic as well as many other biologic radiation effects can possibly be ascribed to changes in desoxyribonucleic acids (DNA) or their complexes with proteins. The polysaccharides might therefore seem to constitute systems, which are chemically too unrelated to the biological materials mentioned to permit drawing any conclusions. The influence of the moisture content on radiation sensitivity seems to be a general phenomenon, however, also being at work for certain changes in proteins (unpublished) and DNA (after UV irradiation¹⁵). The possible significance of the present results for biological effects might therefore be discussed on general terms.

Different chemical effects induced in the polysaccharides depend differently on the water content: Some effects, as the degradation measured spectrometrically or viscosimetrically, are greatest at low water contents and are inhibited by the presence of water quantitatively to the same degree as the biological effects. The degradation of amylopectin irradiated at different water contents was followed also in pure nitrogen atmosphere, and quantitatively identical results were obtained; in the biologic material water exerts the same protective influence in nitrogen (which, in addition, causes some protection).¹ From the similar displacement of spectra after irradiation and enzymatic hydrolysis it does not seem plausible that the degradation measured can be ascribed to a breaking of great numbers of hydrogen bonds¹⁶. Chemical changes involving rupture of the polysaccharide chains are certainly at work. That chemical changes involving covalent bonds do follow the degree of degradation is further indicated by the ability of irradiated starch to reduce iodine in solution, the ionic yield for this ability decreasing from about 0.5 at 6 % H_2O to 0.2 at 20 % H_2O . (The chemical nature of the iodine consuming groups is being studied further.) Other reactions show the inverse dependence on the water content, especially the oxidation of adsorbed iodide¹¹ and possibly also the induction of acid groups at high water contents. Still other reactions are independent of the water content, such as the rupture of glycosidic bonds, probably also the induction of acid groups below 16 % H_2O . The occurrence of these different modes of reaction gives, in principle, means to elucidate the chemical changes, leading to moisture-dependent biologic radiation effects.

The validity of the estimations of the number of chain breaks per ion pair is strengthened by the quantitative agreement between the two very different

systems: amylose, *i.e.*, an unbranched polysaccharide, irradiated with 10—24 Mr, and the branched amylopectin irradiated with a 100 times lower dose. It is therefore reasonable to assume that per ion pair, *i.e.*, 32.5 eV absorbed, about 5 breaks occur when the material is irradiated at about 6 % H₂O. Taking into consideration the many experimental difficulties and possible sources of error, the latter value can be said to be certainly higher than 3 breaks per ion pair and accordingly greater than the yields of acid groups or glucosidic bonds hydrolyzed. An increase of the water content to about 20 % at irradiation decreases the yield of chain breaks by 2—5 times, in similarity with the biological experience, bringing it to a value close to one break per ion pair. The high ionic yield obtained when irradiating the desiccated material may be taken as an indication that excitations are chemically effective here. — The possible biologic importance of excitations has but infrequently been discussed. As a rule they have been regarded unimportant (*cf.* Lea¹⁷).

Due to difficulties in measuring the number of excitations in materials such as those we are considering here, the frequency at which they occur is still a matter of dispute. For various materials values amounting to 1—3 excitations per ion pair have been given¹⁶⁻¹⁸. If all of them were leading to chemical reactions, ionic yields amounting to 2—4 would be obtained. We have therefore some justification to suppose, at present as a working hypothesis, that in biologic materials as well as in non-living model systems, water exerts its maximum "protection" at high water contents (about 20 % H₂O) by eliminating the chemical rearrangements following excitation, whereas the chemical consequences of ionization are not affected to the same degree. Regarding the number of excitations per ion pair this would mean a dose reduction factor of 2—4, in agreement with the experiments. The idea is further supported by the fact that Kaplan¹⁵, irradiating by UV bacteria as well as DNA at different water contents, finds a dose reduction factor, in both systems amounting to about 22. The low energy of the UV-photons makes the frequency of ionizations relatively much more rare, and, consequently, a protection against excitation leads to a much more pronounced "protective effect" than in the case of ionizing radiations.

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