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Dye-Sensitized Photopolymerizations of Acrylic Monomers in the Presence of Fibrous Proteins

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Synopsis

Dye-sensitized photopolymerizations of low concentrations (1–5%) of acrylic monomers proceed at 40°C., if fibrous proteins (keratin, fibroin or collagen) are suspended in solution. In the absence of dye (riboflavin or fluorescein) or fibrous protein no appreciable photopolymerization of monomer is observed at the short irradiation times (120 min.). Graft products containing 0.5–23% polymer are found. The quantity of grafted polymer introduced into the protein depends on the chemical nature and concentration of monomer, dye, and fibrous protein. Homopolymerization of monomer is observed to some extent in each graft polymerization. In the presence of oxygen an induction period is found, but removal of oxygen from solution greatly retards these photopolymerizations. Chemical and physical characterization of the protein–polymer products suggests that only a small number of initiation and grafting sites are present in the protein and that the grafted polymer partially resides within the protein matrix. We believe these photopolymerizations proceed via a free-radical pathway involving radical abstraction of hydrogen from the protein by dye intermediates.

Dye-sensitized photopolymerizations of moderate to high concentrations (>10%) of aqueous monomers in the presence of oxygen have been studied extensively[1–10] in recent years. Whereas riboflavin-sensitized photopolymerizations require only oxygen in solution, other dye-sensitized photopolymerizations[1,3–6,8–10] require added reducing agent in addition to oxygen. Since the rate of dye-sensitized photopolymerization is proportional to the square of the monomer concentration,[2,7,8] photopolymerizations of low concentrations of monomer (1–3%) proceed very slowly, and no appreciable polymerization occurs over short irradiation times (1–4 hr.).

Recently we found that riboflavin-sensitized photopolymerizations of low monomer concentrations proceed after short irradiation times,[11] if proteins are dissolved or suspended in the monomer solutions. Furthermore, we find that dyes that normally require added reducing agent (i.e., eosin, fluorescein) cause photopolymerization of monomer in the presence of proteins, even without added reducing agent.

EXPERIMENTAL

Proteins and Reagents

Wool keratin was extracted with ethanol for 8 hr. and with ether for 8 hr. prior to use. Shredded and lyophilized collagen (bovine achilles tendon) and silk fibroin[12] were obtained from General Biochemicals, Inc. All proteins were brought to constant weight at 21°C. and 65% R.H. before and after use. Monosodium riboflavin-5′-phosphate (Calbiochem), disodium fluorescein (J. T. Baker), and acrylic monomers (Eastman) were used. All liquid monomers were distilled immediately before use.

Photopolymerization of Acrylic Monomers in the Presence of Fibrous Proteins

An aqueous solution (100 ml.) containing 0.1–10 mg. of dye, 1–5% acrylic monomer, and 2–3 g. of fibrous protein were divided and placed in two Pyrex test tubes 25 mm. in diameter. The tubes were placed in a Pyrex water-filled bath maintained at 40.0 ± 0.1°C. The solutions were irradiated from 10 cm. distance with a 275 w. Westinghouse RS sunlamp[13] (emission maxima at 365, 405, 436 mμ). After 2 hr. of irradiation the fibrous proteins were removed from solution and washed for 20 min. in 60°C. water and then with distilled water. The samples were dried, brought to constant weight, and weighed. The viscosities of the solutions remaining in the test tubes were measured in a Cannon-Fenske, size 100, viscometer. Water-insoluble homopolymers were precipitated by addition of sodium chloride to solution. In no case did the amount of homopolymer exceed 50% polymerization of the initial monomer. Data for the photopolymerizations are found in the tables, and each polymerization result is the average of four or more runs.

Gas–liquid chromatography (GLC) of aliquots from selected reaction mixtures indicates that rapid polymerization takes place after a 30–60 min. induction period. Photopolymerization proceeds so rapidly that the change of monomer concentration with time cannot be followed readily by GLC; however, GLC shows that no more than 40–60% conversion of monomer occurs in any run.

Analytical Methods

Nitrogen analyses were performed by the Kjeldahl procedure. Amino acid analyses have been described previously.[12] After allowance was made for the polymer present in the products, it was found that no significant change in amino acid contents of the samples had occurred.

The total incident light intensity I_0 was determined by the actinometric procedure of Parker and Hatchard.[14,15] The colorimetric measurements were made on a Perkin-Elmer spectrophotometer, Model 202 ($I_0 = 1.7 \times 10^{-6}$ ein./hr./ml.).

Fibers from the photopolymerizations were compared with fibers that contained homopolymers deposited from solution and with control samples.

The fibers were examined under a Leitz Dialux-pol polarizing microscope to establish the nature of polymer on the fibers. The change of damping constant Δ with temperature was determined for 100–200°C. with a torsion pendulum assembly by the procedure of Menefee and Yee.[16] The samples were heated at 100–110°C. in a vacuum for 2 hr. prior to determination of Δ.

Papain digestion of the keratin–polymer products was by the procedure of Lennox and Forss.[17] Fibroin was dissolved from the fibroin–polyacrylamide product with the use of 50% lithium bromide.[12]

RESULTS AND DISCUSSION

Riboflavin-Sensitized Photopolymerizations of Monomers in the Presence of Wool Keratin

Riboflavin-sensitized photopolymerizations of several water-soluble monomers proceed in the presence of keratin during 2 hr. of irradiation; see Table I. After an induction period of 30–60 min. rapid photopolymerization takes place, and the uptake of grafted polymer is dependent on the nature of the monomer in solution.

Methyl acrylate gave the highest uptake of polymer, acrylamide and *N,N*-dimethylacrylamide gave intermediate uptakes, and acrylonitrile, acrylic acid, and *N*-vinylpyrrolidone gave low uptakes. Differences in graft uptakes cannot fully be explained by differences in reactivity of these monomers, and other factors, such as the permeability of keratin by the various monomers and the interaction of dye and monomer, must have an effect on the grafting of polymer to keratin.

In each case photopolymerization is accompanied by significant homopolymerization of monomer in solution. In cases in which the homopoly-

TABLE I
Riboflavin-Sensitized Photopolymerizations of Acrylic Monomers in the Presence of Keratin[a]

Monomer	Concn., g. per 100 ml.	Polymer in grafted product,[c] %	Final η_{rel} / init. η_{rel}	N, % Calcd.	N, % Found
—	—	—	1.00	—	16.5
Acrylamide	3.0	10.1 ± 1.4	7.26 ± 1.71	16.8	16.8
Acrylamide[b]	"	3.4 ± 0.5	1.28 ± 0.07	16.5	16.5
N,N-Dimethylacrylamide	"	8.8 ± 0.7	11.8 ± 1.01	16.3	16.2
N-Vinylpyrrolidone	"	0.8 ± 0.6	1.04 ± 0.01	16.3	16.4
Acrylic acid	"	4.3 ± 0.8	1.14 ± 0.05	15.8	15.8
Methyl acrylate	"	22.5 ± 1.8	—	12.8	12.3
Acrylonitrile	"	5.0 ± 0.2	—	17.0	17.2

[a] Monosodium riboflavin-5′-phosphate concentration, 1.0 mg. per 100 ml.
[b] Nitrogen bubbled through the solution for 30 min. prior to irradiation.
[c] Weight per cent grafted polymer in keratin–polymer product.

mers are water-insoluble less than 10% homopolymer formation is found; however, as much as 50% homopolymerization is found with water-soluble polymers. Homopolymerization apparently does not result from simple dye-sensitized photopolymerization of monomer, since no homopolymer is formed in the absence of keratin. Homopolymerization is apparently initiated on chain termination of the growing, grafted polymer chain by hydrogen abstraction, giving new radical species capable of initiation of homopolymerization.

When polymerization occurs in these systems after the induction period, it is rapid, and the reaction is essentially complete within 15–30 min. Although oxygen contributes to the observed induction period, its removal from these systems greatly retards photopolymerization, as previously observed.[1,3–8]

The riboflavin–oxygen initiating system possesses a low initiating radical efficiency,[1,2,8,9] demonstrated by the high molecular weight of polymers from the photopolymerizations. In our studies the high viscosities of some of the homopolymers and unchanged amino acid contents in the protein–polymer products are consistent with a low radical initiating efficiency. The complexity of the reaction system prevents a complete characterization of the initiation mechanism. However, in light of past studies and our results, we believe initiation of these photopolymerizations proceeds via a free-radical pathway involving radical abstraction of hydrogen from the protein by previously proposed[2,8,9] dye intermediates.

Effect of Acrylamide and Riboflavin Concentration on Photopolymerization

The concentrations of acrylamide and riboflavin were varied in order to determine their effect on the photopolymerizations; see Table II. When the acrylamide concentration was lowered to 1%, only limited photopoly-

TABLE II
Effect of Change of Riboflavin and Acrylamide Concentrations on Photopolymerizations in the Presence of Keratin

Acrylamide concn., g. per 100 ml.	Riboflavin concn., mg. per 100 ml.	Polymer in grafted product,[a] %	Final η_{rel} / init. η_{rel}	N, % Calcd.	N, % Found
—	—	—	1.00	—	16.5
—	1.0	—	—	—	16.5
3.0	—	0.4 ± 0.4	1.06 ± 0.02	16.5	16.6
1.0	1.0	0.5 ± 0.3	1.16 ± 0.03	16.5	16.4
3.0	1.0	10.1 ± 1.4	7.26 ± 1.17	16.8	16.8
5.0	1.0	6.3 ± 0.3	≫1000	16.7	16.8
3.0	0.1	3.1 ± 0.5	18.2 ± 3.4	16.6	16.7
3.0	10.0	0.0 ± 0.1	1.36 ± 0.08	16.5	16.5

[a] Weight percent of grafted polyacrylamide in keratin–polyacrylamide product.

merization was observed; however, when a 5% acrylamide solution was used, grafting to the protein was lower than for 3% acrylamide solutions, although the viscosity of the solution was dramatically increased, suggesting increased homopolymerization. It appears that the concentration of acrylamide suitable for graft polymerization goes through a maximum between 1 and 5% monomer, the homopolymerization increasing at higher monomer concentrations.

A tenfold decrease in the riboflavin concentration results in significantly lower polymer grafting and increased solution viscosity, whereas no polymer grafting is found when the dye concentration is increased by a factor of 10. Changes in riboflavin concentration have an effect similar to that of changes in acrylamide concentration, since either change has the same net effect on the ratio of riboflavin to acrylamide.

Fluorescein-Sensitized Photopolymerizations

Riboflavin was initially chosen for the photopolymerizations because the dye possesses absorption maxima near the 365, 405, and 436 mμ lines of the RS sunlamp, and because it possesses a mild reducing sugar moiety essential for photopolymerization in the absence of protein. Disodium fluorescein was chosen as a dye that did not possess a mild reducing function but at the same time absorbed significant quantities of light at the given wavelengths. Although the two dyes are not strictly comparable, they do provide data that will show the effect of the reducing function on the photopolymerizations; see Table III.

Fluorescein-sensitized photopolymerizations occur in the presence of proteins without addition of reducing agent. Apparently the side chains of the protein provide the mild reducing function necessary for the photopolymerization to proceed. Fluorescein generally gives lower polymer grafts than riboflavin; however, higher grafts are observed for photopolymerization of acrylic acid, probably owing to the effect of higher acidity (pH ≈ 1) of solution on the electronic structure of the dye.

TABLE III
Fluorescein-Sensitized Photopolymerizations of Acrylic Monomers in the Presence of Wool Keratin[a]

Monomer	Concn., g. per 100 ml.	Polymer in grafted product,[b] %	Final η_{rel} / init. η_{rel}	N, % Calcd.	N, % Found
—	—	—	1.00	—	16.5
Acrylamide	3.0	4.9 ± 0.2	1.22 ± 0.04	16.7	16.6
Acrylic acid	"	10.2 ± 0.5	1.11 ± 0.04	15.0	15.0
Methyl acrylate	"	15.4 ± 2.0	—	14.3	13.8

[a] Disodium fluorescein concentration, 1 mg. per 100 ml.
[b] Weight per cent grafted polymer in keratin–polymer product.

Furthermore, fluorescein-sensitized photopolymerizations cause very little homopolymerization, in contrast to riboflavin-sensitized photopolymerizations. This may be attributed in part to the lack of a reducing moiety in fluorescein, thereby making it less likely to cause homopolymerization side reactions.

Photopolymerizations in the Presence of Silk Fibroin and Collagen

Riboflavin-sensitized photopolymerizations of acrylamide in the presence of fibroin and collagen (Table IV) give grafts of polymer comparable to those found for keratin. Significant homopolymerization is also observed. Grafting of methyl acrylate was much lower for fibroin than for keratin, demonstrating that grafting is highly dependent on the fibrous protein present in solution.

TABLE IV
Riboflavin-Sensitized Photopolymerizations of Monomers in the Presence of Silk Fibroin and Collagen[a]

Protein	Monomer	Concn., g. per 100 ml.	Polymer in grafted product,[b] %	Final η_{rel} / init. η_{rel}	N, % Calcd.	N, % Found
Fibroin	—	—	—	1.00	—	17.3
Fibroin	Acrylamide	3.0	8.6 ± 1.3	3.22 ± 1.50	17.5	17.7
Fibroin	Methyl acrylate	3.0	2.5 ± 0.6	—	16.9	16.9
Collagen	—	—	—	1.00	—	16.4
Collagen	Acrylamide	3.0	10.9 ± 1.6	10.85 ± 1.41	16.7	16.6

[a] Monosodium riboflavin-5′-phosphate concentration, 1.0 mg. per 100 ml.
[b] Weight per cent graft polymer in protein–polymer product.

Characterization of Protein–Polymer Products

The fibers from these photopolymerizations (Tables I–IV) were characterized as follows. The percentage nitrogen in each of the resulting fibers compared favorably with the percentage nitrogen content calculated from the observed uptake of polymer. All attempts to extract further homopolymer from polymer–protein fibers with hot solvents for the homopolymers failed. Attempts to isolate the grafted polymer from wool by digestion of the wool with aqueous papain[17] were unsuccessful, since extensive hydrolysis and degradation of the polymer occurred even under these mild reaction conditions. Silk fibroin was dissolved away from fibroin–acrylamide copolymer with 50% lithium bromide, yielding 10% polyacrylamide.

The protein–polymer products from the photopolymerizations were compared under a polarizing microscope with samples of fibrous proteins that contained similar contents of homopolymers deposited from solution. The protein–polymer products were observed to have an uneven coating

of polymer on the exterior of the fiber, the polymer appearing as small nodes along the fiber. Fibers that contained similar amounts of polymer deposited on the fiber from solution appeared to have higher concentrations of polymers on the fiber surface. These observations suggest that in the photopolymerizations some graft photopolymerization of monomer occurs within the wool fiber.

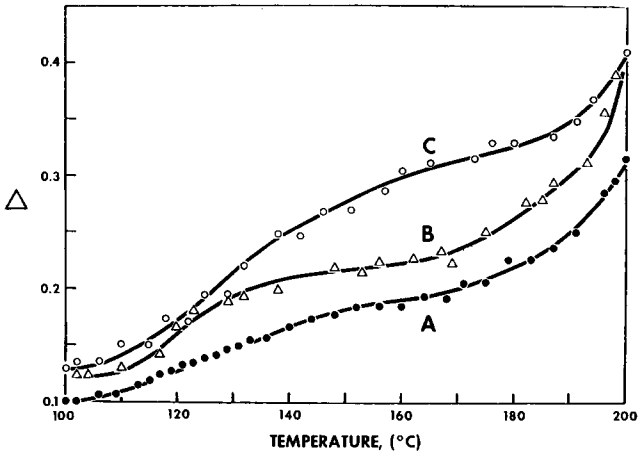

Fig. 1. Change in damping constant Δ with temperature for control and keratins treated with methyl acrylate: (A) control, 2 hr. irradiation in 1 mg. per 100 ml. of disodium riboflavin-5′-phosphate; (B) keratin containing 22.5% photopolymerized methyl acrylate; (C) keratin containing 13.2% poly(methyl acrylate) deposited from tetrahydrofuran.

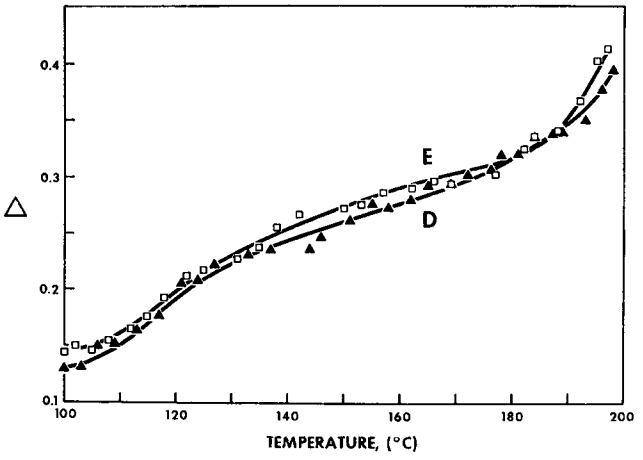

Fig. 2. Change in damping constant Δ with temperature for keratins treated with polyacrylonitrile: (D) keratin containing 4.9% photopolymerized acrylonitrile; (E) keratin containing 6.6% polyacrylonitrile deposited from *N*,*N*-dimethylformamide.

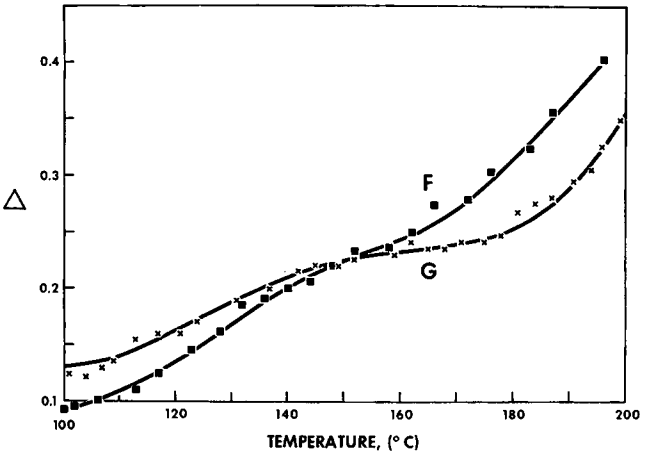

Fig. 3. Change in damping constant Δ with temperature for keratins treated with polyacrylamide: (F) keratin containing 10.1% photopolymerized acrylamide; (G) keratin containing 13.6% polyacrylamide deposited from water.

To establish that internal deposition of polymer occurs to some extent during photopolymerization and that a real difference exists between the grafted photopolymerization products and fibers on which ungrafted polymer is deposited, the change in the damping constant Δ with temperature[16] for selected fibers was determined; see Figures 1–3.

The damping constant Δ is a gage of the internal friction of a fiber and is independent of the fiber geometry,[16] and fibers that contain polymer give higher damping constants over the temperature range than control keratins. Furthermore, polymer deposited on the exterior of the fiber will have a greater effect on Δ than polymer distributed through the fiber.[18]

The change in damping constant with temperature (Fig. 1) for methyl acrylate photopolymerization on keratin (B) falls between the values for control keratin (A) and keratin containing deposited polymethyl acrylate (C). The change in damping constant with temperature for photopolymerized acrylonitrile on keratin (D) is only slightly lower than for polyacrylonitrile deposited on keratin (E), possibly because of the difficulty of detecting small differences in the fibers at these lower uptakes. Wool that contains deposited polyacrylamide (Fig. 3, G) has higher damping constants than photopolymerized acrylamide on wool (F) from 100 to 150°C., but from 150 to 200°C. (G) possesses significantly lower damping constants. This change is probably due to crosslinking of the outer polyacrylamide layer in the case of deposited polymer (G), forming imide crosslinks and thereby increasing the rigidity of the wool fiber. Polyacrylamide distributed within the fiber will be less likely to give crosslinking.

Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

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