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Some Applications of Fluorimetry to Synthetic Polymer Studies

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Summary. Fluorimetry, a powerful research tool in the study of biological macromolecules, has a number of valuable applications in investigations of synthetic polymers. These techniques reveal the microscopically heterogeneous distribution of species in polymer systems, which is specially pronounced in solutions of polyelectrolytes. They also allow us to study conformational mobility of flexible chains, diffusion-controlled intermolecular reactions of macromolecular reagents, glass transition phenomena, and polymer compatibility.

Fluorimetry has long been used in studies of biological macromolecules. For instance, dyes whose fluorescence yields are sensitive to the polarity of the medium (*1*, *2*) can be used as "reporters" to characterize the polarity of the specific protein sites to which they are adsorbed or covalently attached. Fluorescence may then be used to monitor protein denaturation (*2*, *3*) and even small conformational changes such as those accompanying the formation of chymotrypsin from its precursor chymotrypsinogen (*4*). Depolarization of the fluorescence of chromophores that are rigidly attached to globular proteins may be used to calculate the rotational diffusion constants of the proteins (*5*, *6*) and the change in the rotational diffusion constant may be used to follow the kinetics of association of two proteins, such as an antigen and its antibody (*7*).

More information is available when the decay in the emission anisotropy is followed as a function of time after a short light flash. For instance, in a solution containing an antibody associated with a fluorescent hapten the decay was found to be biphasic, and this was interpreted as reflecting a flexible "hinge" in the antibody structure (*6*). In some proteins containing a single fluorescent tryptophan residue the intensity of the fluorescence, excited by a light flash, does not follow simple exponential decay. This was considered to show that the proteins have a certain degree of conformational mobility, so that the tryptophan residues in different molecules are in different local environments during the lifetime of the excited state (*8*).

Energy absorbed by a chromophore may be transferred to a second chromophore up to 5 nanometers away if the emission spectrum of the donor overlaps the absorption spectrum of the acceptor. The theory of this nonradiative energy transfer was formulated by T. Förster and its applications to biochemistry were reviewed by Steinberg (*9*). This phenomenon is responsible for the fact that emission spectra of proteins containing phenylalanine, tyrosine, and tryptophan residues exhibit only the band characteristic of tryptophan (*10*). Since the extent of nonradiative energy transfer is a sensitive function of the distance separating the donor and acceptor chromophores, the phenomenon can be used to measure their separation. This principle was used to determine the distance between Tb(III) and Co(II) ions bound to specific sites in the enzyme thermolysin, and the estimate obtained was found to be in good agreement with its known molecular structure (*11*). Other applications of fluorescence to the study of biological macromolecules are reviewed in (*12*).

Fluorescence techniques have had more limited use in the study of synthetic polymers. In this article, although I will not review all of the work done in this area, I will try to show what a wide variety of questions can be illuminated by fluorimetry.

Fluorescence Quenching

In systems containing only low molecular weight species, the relation between the fluorescence intensities I and I_0 in the presence and absence of a quencher at concentration Q is commonly expressed by the Stern-Volmer equation $I_0/I = 1 + K_{SV}(Q)$. This simple relation is valid if quenching involves collisions of the excited chromophore with the quencher, and K_{SV} is then the ratio of the rate constants for the chromophore-quencher reaction and for emission from the excited chromophore. In solutions of macromolecules complications may arise as a result of a microscopically nonuniform distribution of the fluorescent species, the quencher, or both. Weill and co-workers studied the behavior of solutions containing a small fluorescing species and a polymer with quenching groups attached at random along the backbone of the polymer chain (*13*) and the behavior of solutions containing small quenching molecules and a fluorescing polymer (*14*). Both types of systems are characterized by Stern-Volmer plots that deviate markedly from linearity. In the first case the system may be thought of as consisting of two regions, one representing the polymer domains with a high quencher concentration, the other outside the polymer coils with no quenchers. The dependence of the fluorescence intensity on the concentration

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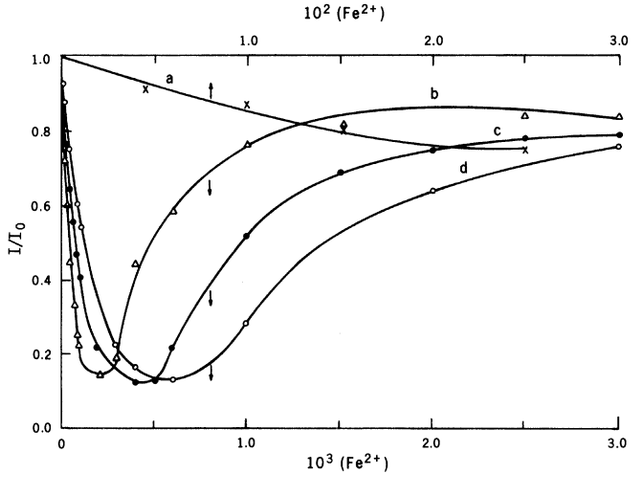


Fig. 1. Effect of PVS on the quenching of the fluorescence of quinine cations by Fe^{2+}: (a) no PVS, (b) $10^{-3}N$ PVS, (c) $2 \times 10^{-3}N$ PVS, and (d) $3 \times 10^{-3}N$ PVS.

of the polymeric quencher may be interpreted in terms of a critical concentration at which the polymer chains begin to be sufficiently entangled so that the system has a uniform quencher concentration. In the second case, deviation from a linear Stern-Volmer plot is due to a difference between the solvation of the fluorescing polymer by the principal solvent and its solvation by the quenching agent. The fluorescence data could be interpreted quantitatively in terms of the extent to which the quencher was concentrated in the polymer domain or excluded from it.

Effects due to an unequal distribution of interacting species are particularly dramatic in polyelectrolyte solutions, where there are large energetic interactions between the polyion and small charged species. For instance, Fe^{2+} quenching of the fluorescence of UO_2^{2+} (*15*) and of the doubly charged quinine cation (*16*) has been studied in the presence and absence of the polyanion polyvinylsulfonate (PVS). At low Fe^{2+} molarities, (Fe^{2+}), the polyanion increased the fluorescence quenching by a large factor, but increasing (Fe^{2+}) led eventually to a spectacular increase in the fluorescence intensity (Fig. 1). This is because at low Fe^{2+} concentrations the fluorescing species and the quencher are both concentrated in the polymer domain and the quenching efficiency is greatly increased. [This effect is analogous to the catalytic effect of polyanions on other reactions involving two cationic species (*17*).] However, as the concentration of the quencher is increased it eventually displaces the fluorescent species from the polymer domain into a region where the quencher concentration is very much lower, leading to an enhancement of fluorescence intensity.

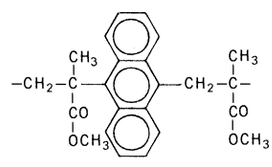
Similar phenomena were observed with the quenching of the ruthenium bipyridyl complex $Ru(bpy)_3^{2+}$ fluorescence by Cu^{2+} in the presence of PVS (*18*). As would be expected, a polyion inhibits reactions between positively and negatively charged species—for instance, between solvated electrons and cations (*19*). A particularly interesting effect has recently been demonstrated: when $Ru(bpy)_3^{2+}$ is excited in the presence of a neutral electron acceptor, the back reaction that takes place normally within the solvent cage may be efficiently inhibited by PVS, which attracts the resulting $Ru(bpy)_3^{3+}$ but repels the anionic product of the electron transfer (*20*).

Fluorescence quenching is generally a diffusion-controlled process. Thus, if a polymer carries both fluorescing and quenching chain substituents, intramolecular quenching is governed by the rate of conformational transitions of the polymer chain, which allow the fluorescing and quenching groups to diffuse toward each other. Experiments of this type were first reported by Kirsh *et al.* (*21*), who used polymers carrying fluorescing and quenching groups distributed at random along the chain. However, they discussed the experimental data in terms of an "effective local concentration c_{eff} within the polymer coils," which is misleading since an averaging of the polymer segment concentration within some effective volume of the randomly coiled polymer would lead to decreasing values of c_{eff} with an increasing length of the polymer chain. A treatment taking account of the connectivity of the chain must lead to c_{eff} rapidly approaching an asymptotic value (*22*).

When a solution contains two similar polymers, one carrying fluorescing groups and the other carrying quenching groups, the change in fluorescence intensity as the concentration of the quenching polymer is changed reflects the ability of the polymeric coils to interpenetrate one another. Kirsh *et al.* (*21*) showed in an experiment of this type that interpenetration of the flexible chain molecules is negligible until a critical polymer concentration has been attained. Since no such threshold was observed in intermolecular polymer reactions characterized by a high activation energy (*23*), the effect reported by Kirsh *et al.* is apparently restricted to diffusion-controlled processes involving species with very short lifetimes, such as excited chromophores. Intermolecular polymer reactions involving the interaction of phosphorescent groups and quenching groups attached to the ends of polystyrenes with very narrow chain length distributions (*24*) are apparently governed by segmental diffusion of the interacting groups to the surface of the polymer coils; the rate constant of this process was found to be inversely proportional to the 0.32 power of the chain length.

Depolarization of Fluorescence

When a fluorescent group is attached to the end of a flexible polymer chain, its fluorescence is depolarized less than that of the free chromophore, reflecting its increased resistance to rotational diffusion. As would be expected, the apparent rotational diffusion constant of the chromophore is sensitive to the length of the chain to which it is attached only for relatively short chains; with polyacrylamide chains a limiting value is reached for polymers containing about 200 monomer residues (*25*). Data of this type are related to the conformational mobility of polymer chains, and they can be more reliably interpreted if the chromophore is attached rigidly to a very long chain. In this case, the rotational diffusion of the macromolecule as a whole is much slower than local conformational transitions, and the depolarization of fluorescence may be interpreted in terms of the conformational mobility of the polymer chain backbone. This approach was used with poly(methyl methacrylate) with anthracene residues incorporated into the chain backbone (*26*) (**1**) and with polystyrene containing a small number of 9-*p*-vinylphenyl-10-phenylanthracene residues



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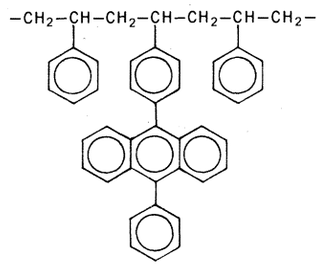
(*27*) (**2**). One interesting feature of the results obtained was the low value of the apparent activation energy for the conformational transitions: 2 to 3.4 kilocalories per mole. This is no more than



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would be expected for low molecular weight analogs of these polymers, and I will return later to the significance of this finding.

When a polymer chain undergoes a cooperative conformational transition (where the conformational change in one monomer facilitates a similar transition in neighboring residues), the process can be monitored by following the change in the depolarization of fluorescence of a chromophore attached to the chain backbone. Sharp changes in emission anisotropy were observed when the ionization of poly(methacrylic acid) passed through the range in which the chain changes from a compact conformation to a highly expanded coil (*28*) and during the helix-coil transition of a polypeptide (*29*).

More detailed information can be obtained if the decay of the fluorescence and its polarization are followed after a short flash of exciting radiation. Such a study was reported by Valeur and Monnerie (*30*) for compound **2** as well as for a polystyrene with a 9,10-diphenylanthracene residue incorporated in the chain backbone. In both cases the time dependence of emission anisotropy deviated from simple exponential decay. The mean relaxation time computed from this time dependence was almost linear in the viscosity of the medium, although a small downward curvature was observed in the low-viscosity range. This behavior is consistent with general theories of chemical processes in viscous media, which predict that reciprocal rate constants will be linear in viscosity (*31–33*).

The interpretation of the decay of the emission anisotropy meets with mathematical complications since the duration of the exciting light flash is not negligible compared to the lifetime of the excited state. In addition, there are experimental limitations to the study of the time dependence of emission anisotropy. However, it has been shown that equivalent information may be obtained more easily by steady-state measurements of the emission anisotropy with the excited lifetime of the chromophore varied by addition of quenching reagents (*34*).

Excimer Fluorescence

A number of excited aromatic molecules may associate with a similar molecule in its ground state to form a sandwich complex in which the two aromatic residues lie parallel to each other at a distance of about 3 angstroms. Such "excimers" are characterized by a broad unstructured emission which is shifted to longer wavelengths with respect to the emission spectrum of the monomer (*35*). Analogous excimer formation may take place by an intramolecular process in molecules carrying two similar chromophores, such as 1,3-diphenylpropane (*36–43*). In this case, it is clear that the conformation required for excimer formation would correspond to a prohibitively large potential energy if the aromatic residues were in their ground states. We must therefore assume that the intramolecular excimer can form only if a conformational transition that brings the two chromophores into proper juxtaposition takes place during the lifetime of the excited state. This assumption has been verified both by time-resolved fluorescence decay techniques (*39, 40*) and by finding that excimer formation is inhibited in media of high viscosity (*40, 42, 43*). The relative quantum yields of excimer and monomer fluorescence can be interpreted in terms of the ratio of the rate constants for conformational transition and for emission from the monomer, and this provides a method for measuring rate constants for conformational transitions in the range of 10^7 to 10^9 sec^{-1}.

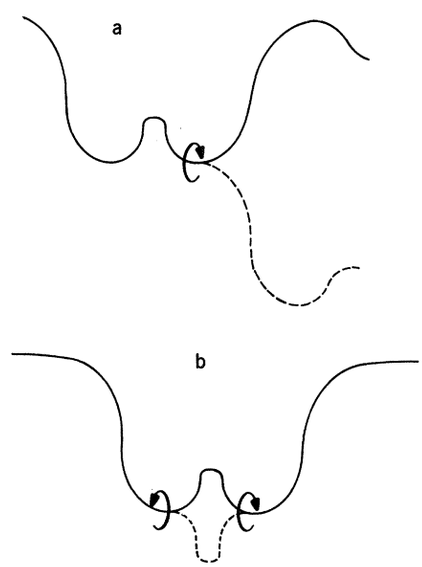


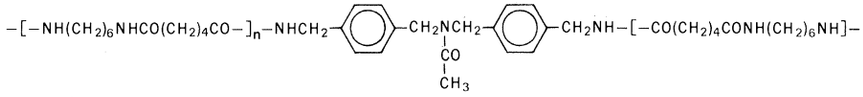
Fig. 2. Schematic representation of conformational transitions in polymer chains. (a) A single rotation around a bond in the chain backbone. (b) Two correlated rotations in a crankshaft-like motion.

This method is particularly valuable to the polymer chemist. If we consider conformational transitions of a flexible chain molecule in dilute solution, we face a conceptual difficulty, which is illustrated schematically in Fig. 2. If the process is restricted to hindered rotation around a single bond of the chain backbone, a large portion of the chain would have to swing through the viscous medium with a prohibitively large dissipation of energy. It has therefore been suggested that two conformational transitions are correlated in time, so that only a short segment of the chain has to move by a "crankshaft-like motion" (*44*). Although this concept was originally advanced to interpret conformational transitions of polymers in bulk, it has been widely used in theoretical treatments (*45–47*) and in the interpretation of experimental results obtained with flexible polymers in dilute solution (*48*). But the activation energy for two strictly simultaneous conformational transitions within the polymer backbone would be twice as large as the activation energy for an analogous small molecule (*44, 47, 49*), and even if the requirement for simultaneity were somewhat relaxed, the activation energy should still be substantially larger in the polymers. This appears to contradict a number of experimental results (*26, 27*) that led to activation energies in the range 2 to 3.4 kcal/mole—no larger than those found for liquid butane (*50*) or for 2,4-diphenylpentane, the low molecular weight analog of polystyrene (*51*).

To resolve this apparent contradiction it is necessary to compare the observed rates of conformational transitions around a particular bond in a polymer backbone and around a similar bond in a small molecule. The conformational mobility of polymers has been studied by a number of experimental methods, including the measurement of dielectric dispersion, fluorescence emission anisotropy, nuclear magnetic resonance relaxation, and the broadening of the electron spin resonance spectra of spin labels (*52*). However, none of these methods can be used for a comparison of polymers and their analogs, since the effects that are observed by these techniques in solutions of small molecules are dominated by rotational diffusion of the solute. In principle, sound absorption mea-

surements should yield rates of conformational transitions for both small and large molecules, but the interpretation of the polymer data is uncertain. For example, the activation energies for the meso and racemic forms of 2,4-diphenylpentane were determined by sound absorption as 3.2 ± 1.7 and 3.1 ± 0.5 kcal/mole (*51*), while data on polystyrene samples with molecular weights of 4000 and 97,000 yielded activation energies of 4.0 and 8.3 kcal/mole (*53*). This implies that the conformational mobility close to the chain ends of polystyrene is similar to that of the low molecular weight analog but that in the middle of long chains it is very much reduced.

Excimer fluorescence has been observed in dilute solutions of polymers such as polystyrene (*54*), polyvinylnaphthalene (*55*, *56*), and poly(*N*-vinylcarbazole) (*57*), and it has been shown that excimer formation in these polymers involves the interaction of neighboring aromatic residues along the polymer chain. The process is thus analogous to excimer formation from 1,3-diphenylpropane (*36*), 1,3-dinaphthylpropane (*37*), and 1,3-dicarbazolylpropane (*38*). Nevertheless, it is not possible to compare the relative excimer yield in these polymers and their analogs in a straightforward way in terms of conformational mobility, since migration of the excitation energy along the polymer chains leads to a pronounced increase in excimer fluorescence (*58*). To avoid this complication, we recently prepared a polyamide with widely separated excimer-forming groups in the chain backbone (**3**) and the analogous small molecule (**4**).



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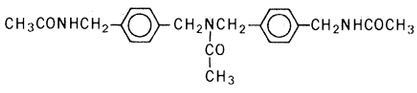

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Figure 3 is an Arrhenius plot of the ratio of the fluorescence intensities of the excimer (I_d) and the monomer (I_m) as a function of temperature for formic acid solutions of **3** and **4**. The slopes of the lines in Fig. 3 cannot be directly interpreted in terms of the activation energy of the conformational transition required for excimer formation, since nonradiative excimer deactivation is also temperature-dependent. However, since one can safely assume that this dependence

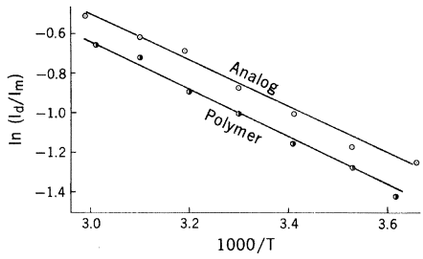

Fig. 3. Arrhenius plot of I_d/I_m for polymer **3** and its analog **4** in 88 percent formic acid.

is the same for the polymer and its analog, the virtual identity of the slopes forces us to conclude that incorporation of the excimer-forming group into the chain backbone has no significant effect on the activation energy of the conformational transition required for excimer formation (*59*). It is difficult to see how this result can be reconciled with the concept of crankshaft-like motions of polymer chains in dilute solution.

Rates of conformational transition are intimately related to the internal viscosity η_i of polymer chains—that is, the resistance to chain end separation (in addition to the resistance provided by the rubberlike elasticity of the chain) in response to a mechanical force applied to the chain ends. Originally, it was believed that η_i is an intrinsic property of the chain, independent of the solvent and dependent solely on the height of the energy barriers $\Delta U^{\ddagger}$ that have to be surmounted as the chain conformation is altered. More recently, however, it has been recognized that conformational transitions involve energy dissipation due to the viscous medium, and it was suggested that the internal viscosity should be of the form $\eta_i = A \exp(\Delta U^{\ddagger}/RT) + B\eta$, where R is the gas constant, T is temperature, η is the viscosity of the solvent, and the parameter B depends on the bulk of the group that has to move through the medium during the conformational change (*60*). We have tested this concept by using data on excimer fluorescence and the lifetime of the excited state to estimate rates of conformational transitions in model compounds such as $ArCH_2OCH_2Ar$, where Ar is a phenyl, 1-naphthyl, or 4-biphenyl group (*43*). As expected, as the bulk of Ar increased, so did the sensitivity of the conformational mobility to the viscosity of the solvent. For the three Ar groups above, rates of conformational transition were estimated to be reduced by a factor of 2 at viscosities of 9.1, 3.5, and 1.2 centipoise, respectively.

Whereas excimer formation from phenyl (*36*), naphthyl (*37*), and carbazolyl (*57*) residues is significant only when these chromophores are separated by three covalent bonds, excimer formation from pyrenyl residues has been observed over a broad range of spacings (*41*). This is due to both the long life of the excited pyrene [about 500 nanoseconds (*35*, p. 179)], which increases the probability that a number of conformational changes will take place, allowing the excited chromophore to encounter a pyrene in the ground state, and the relatively high exothermicity of pyrene excimer formation [about 9 kcal/mole (*35*, pp. 301–371)], which leads to relaxation of the requirement for parallel orientation of the chromophores in the excimer. It has, in fact, been found that intramolecular excimer formation can be observed with poly(ethylene oxide) having pyrene residues at the two chain ends and more than 1000 atoms in the chain backbone (*61*). Since excimer formation is diffusion-controlled, the spectroscopic data may be compared (*62*) with theoretical predictions of the rate at which the polymer chain ends diffuse toward each other (*63*).

Excimer fluorescence was also utilized most effectively for a study of polymers in bulk. When films were cast from solutions of polystyrene containing a small proportion of polyvinylnaphthalene, the films containing molecularly dispersed polyvinylnaphthalene in a glassy polystyrene matrix were found to have values of I_d/I_m that were strongly dependent on the casting temperature (*64*). This was interpreted as reflecting the equilibrium concentration of conformations favorable for excimer formation at the temperature at which the system became rigid. A plot of ln (I_d/I_m) against $1/T$ was linear over a wide temperature range, leading to an estimate of 2500 ± 300 cal/mole for the conformational energy of excimer-forming sites. A later study (*65*) concentrated on the behavior of this glassy solution in the neighborhood of the glass transition temperature T_g of the polystyrene matrix. As the rigidity of the system is reduced, the nonequilibrium conformation of polyvinylnaphthalene can relax to the equilibrium state, and this is reflected by an increase in the excimer fluorescence intensity. The method allowed a kinetic analysis of this process at a temperature as low as 73°C, some 25°C below the T_g for polystyrene. For a film cast at 22°C, the relaxation time was of the order of 100 seconds.

Nonradiative Energy Transfer

Molecules of globular proteins have relatively rigid structures so that the distances between chromophores and their mutual orientation are fairly well defined. It is therefore possible to predict the efficiency of nonradiative energy transfer by a straightforward application of Förster's theory (*9*). However, if chromophores capable of nonradiative energy transfer are attached to the ends of flexible chain molecules, the donor-acceptor distance fluctuates with time. Haas *et al.* (*66*) analyzed the consequences of this fluctuation. They pointed out that after a flash of exciting radiation, energy transfer in molecules with a short end-to-end displacement will be relatively rapid, so that the distribution of end-to-end distances in excited molecules will tend to depart from the equilibrium distribution. The drift toward equilibrium will then depend on the conformational mobility of the chain and will be reflected in the decay of the fluorescence of the donor chromophore. Some caution must be used in interpreting data on nonradiative energy transfer from donor to acceptor chromophores attached to the same chain molecules, since the efficiency of the transfer is highly sensitive to the mutual orientation of the donor and acceptor. Dale and Eisinger (*67*) criticized the assumption that this orientation is random, but the assumption is reasonable if a sufficient number of flexible bonds separate the donor from the acceptor, and it is particularly safe when chromophores with certain spectroscopic characteristics are employed (*66*).

We recently demonstrated that fluorimetry provides a sensitive method for the study of polymer compatibility (*68*). When two polymers are labeled with donor and acceptor chromophores, respectively, and a film is cast from a solution containing a mixture of the two, nonradiative energy transfer from the donor to the acceptor will be small if the two polymers are segregated into two phases, but may be extensive if they form a single phase. The method was tested on a mixture of poly(methyl methacrylate) (PMMA) with copolymers of methyl methacrylate containing increasing concentrations of butyl methacrylate. Surprisingly, there was a gradual transition from the efficient energy transfer characteristic of perfect mixing to a limiting energy transfer efficiency corresponding to complete phase separation when PMMA was mixed with copolymers containing more than 40 mole percent of butyl methacrylate residues. This could reflect a gradual decrease in the depth of mutual interpenetration of the two polymeric species at a "fuzzy" phase boundary, but it could also be interpreted as due to a gradual extension of incompatibility to lower molecular weight fractions of the polydisperse polymers, or to a gradual decrease in the area of the interface as the interfacial energy increased. Future work will have to be designed to differentiate between these possibilities.

Conclusion

I have tried to show the variety of phenomena in polymer chemistry that may be illuminated with the use of fluorescence techniques. These include the microheterogeneity of solutions of uncharged polymers or of polyelectrolytes, conformational mobility and the related problems of the internal viscosity of polymer chains and the diffusion of the ends of flexible chains toward each other, the kinetics of diffusion-controlled intermolecular polymer reactions, the relaxation processes in the neighborhood of the glass transition point, and polymer compatibility. Interested readers may also wish to consult Nishijima's reviews (*56, 69*) of fluorescence methods in polymer science, which are written from a somewhat different point of view.

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Common Origin of Pigment Cells

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Summary. The fundamentally diverse vertebrate pigment cells, melanophores, xanthophores, and iridophores, contain pigmentary organelles known, respectively, as melanosomes, pterinosomes, and reflecting platelets. Their pigments are melanins, pteridines, and purines. Mosaic pigment cells containing more than one type of organelle have been observed and mosaic organelles containing more than one type of pigment have been discovered. It is proposed that the various pigment cells are derived from a stem cell that contains a primordial organelle of endoplasmic reticular origin. This primordial organelle can differentiate into any of the known pigmentary organelles.

The neural crest of vertebrate embryos is intriguing because of the diversity of cell types to which it gives rise (*1*). Among these derivatives are pigment cells, elements of the peripheral nervous systems including the sensory and autonomic ganglia, cartilage, skeletal and connective tissue, and endocrine cells such as the calcitonin-secreting cells of the thyroid and ultimobranchial bodies. It seems implicit from their similar origin that even the most different of these neural crest derivatives must possess common features that set them apart from other elements in the hierarchy of differentiation, but so far none have been identified. In an attempt to approach this problem we have focused our attention on one group of neural crest derivatives, the chromatophores (melanophores, xanthophores, iridophores), which are fundamentally distinct from one another (*2*). It should be emphasized that, while it is generally understood that epidermal melanophores are derived from the neural crest, the same is true for dermal chromatophores including melanophores, xanthophores, and iridophores. Much new information has been obtained about the ultrastructure, chemical composition, and development of these various pigment cells, and this new knowledge has led to a better understanding of how closely the seemingly diverse pigment cells are actually related to one another (*3*). One example of this close relationship is the existence of mosaic pigment cells. The discovery of such cells formed the basis of an hypothesis that the diverse pigment cells of vertebrates originate from a stem cell of neural crest origin (*4*). This is thought to be accomplished by the appropriate differentiation of a primordial organelle into any of the specific pigmentary organelles characteristic of the various pigment cells. Thus, a melanin-containing melanosome, a purine-containing reflecting platelet, or a pteridine-containing pterinosome could be generated to form respectively, melanophores, iridophores, or xanthophores.

In order to fully comprehend the developmental latitude that must be attributed to the stem cell, it is necessary to understand how different are the various pigmentary organelles in both form and composition (Figs. 1 to 5) (*2*). In most vertebrates, melanosomes of both dermal and epidermal melanophores (-cytes) are electron-opaque ellipsoidal structures about 5 nanometers in diameter that usually contain the black-brown insoluble eumelanins. In contrast, melanosomes found in epidermal melanophores in the integument of some birds and mammals, including human red hair, contain phaeomelanin, a lighter colored sulfur-bearing melanin. The phaeomelanosome is about 3 nm in diameter and is somewhat amorphous. Reflecting platelets are the flat-appearing organelles of iridophores. They usually appear as stacks of empty spaces that were previously occupied by purine crystals lost during staining and sectioning. Their purine constituents, notably guanine, hypoxanthine, adenine, and uric acid do not serve as true pigments, but are involved in imparting structural colors. The pigmentary organelles of xanthophores and erythrophores are called pterinosomes. They are spherical in form, about 5 nm in diameter, and contain an internal series of concentric lamellae. It is not known where the monomeric and dimeric pteridines found in these organelles are localized. While the various fully differentiated pigmentary organelles stand out in marked contrast to one another, their early stages of organellogenesis are similar, and they seem to be derived from the endoplasmic reticulum (*3*). In fact, it is this similarity in origin that provides a major part of the concept we are developing concerning the existence of a primordial organelle.

An implicit aspect of our hypothesis concerning the differentiation of the various specific chromatophore types from a stem cell, in response to cues present in the tissue milieu, is that irrevocable chromatophore determination does not take place at the neural crest stage. It is

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