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Intramolecular vs intermolecular formation of bityrosine upon photoreaction of poly(L-tyrosine) in dilute aqueous solution

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Summary

The molecular weight dependence of the rate of the formation of bityrosine upon irradiation of poly(L-tyrosine) at 300 nm has been investigated in dilute aqueous solution, pH 12. The samples studied were the monodisperse monomer, dimer, trimer, and hexamer, as well as two polydisperse polymers with average degrees of polymerization of $\sim 10^2$ and $\sim 10^3$. The reaction is predominantly intermolecular in the monodisperse oligomers, but it is predominantly intramolecular in the two polymers. The intramolecular reaction in the polymers involves phenolic rings on monomers i and $i+j$, with $j > 5$.. The steric restraints imposed by the backbone of the chain make it unlikely that two rings will approach one another with the appropriate geometry for reaction if $j < 5$.

Introduction

The photoreaction of tyrosine that is induced by ultraviolet irradiation has been studied extensively because of its importance in protein chemistry (1–8). The initial step in the reaction is the formation of a phenoxyl radical. Recombination of the radicals can produce various secondary products. One of these secondary products is bityrosine (4). Figure 1 depicts a bityrosine that can be formed by the reaction of two molecules of *N*-acetyl-L-tyrosine-*N*-methyl amide. Ultraviolet irradiation of dilute aqueous solutions of poly(L-tyrosine) produces a strong blue emission that has been recognized as arising from bityrosine (1). This emission can conveniently be measured at 400 nm.

It is of interest to determine whether the intramolecular formation of bityrosine in a polymer is a short-range or long-range event. Are the two tyrosine residues from the same portion of the chain, or might they be widely separated along the contour of the backbone, but brought into close proximity by coiling of the chain? Here we address this question by measuring the initial rates of formation of bityrosine for four monodisperse oligomers (the monomer, dimer, trimer, and hexamer) and for two polydisperse polymers of very different molecular weights.

Materials

All of the tyrosine-containing compounds were purchased from Sigma Chemical Co. The two samples of the polymer were reported by the supplier to have degrees

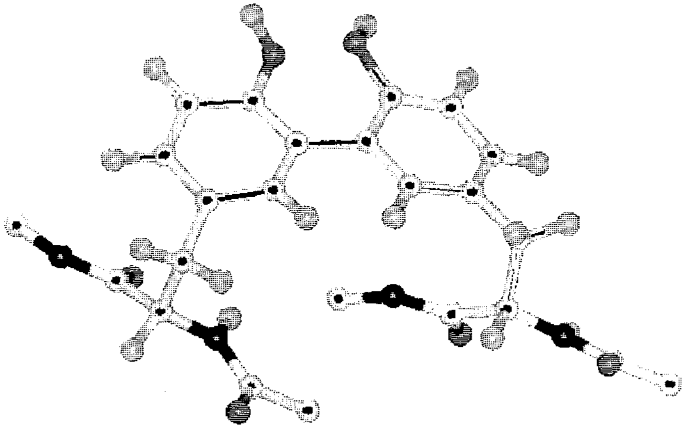

Figure 1. Molecular model of a bityrosine resulting from the reaction of two molecules of N-acetyl-L-tyrosine-N-methyl amide.

of polymerization of 165 and 1376, based on viscosity measurements. The data reported here for the polymer were obtained with the sample of higher molecular weight. Similar results were obtained for the polymer of lower molecular weight. The oligomers were dissolved in 0.01 M NaOH in distilled water. The polymers were dissolved in 0.1 M NaOH and then brought to pH 12.0 by titration with HCl. The solutions studied had a concentration of 10^{-3} M, expressed in tyrosyl units.

The photoreaction was monitored by the intensity of the emission at 400 nm, using front face geometry in an SLM 8000C spectrofluorometer. The light source was a 450 watt ozone-free xenon arc lamp (USHIO UXL450S-O). Double holographic grating monochromators were used to select the incident radiation of 300 nm. Solutions saturated with diatomic nitrogen (N_2) or with nitrogen oxide (N_2O) were prepared by bubbling with the gas for 15 minutes.

Results

When initially prepared, all of the samples exhibited the same features in their excitation and emission spectra. The samples differ in the rate at which irradiation at 300 nm produces a new emission band at 400 nm. The irradiation at 300 nm initiates the photoreaction of tyrosyl units and also excites the fluorescence of any bityrosine present. The new emission band at 400 nm arises from the bityrosine. Its intensity is proportional to the concentration of bityrosine in the solutions (7).

When the majority of the phenoxyl radicals recombine to form bityrosine, the kinetic scheme can be simplified to

$$[A] \xrightarrow{h\nu,k_1} [A\bullet] + e^- \tag{1}$$

$$2[A\bullet] \xrightarrow{k_3} [A-A] \tag{2}$$

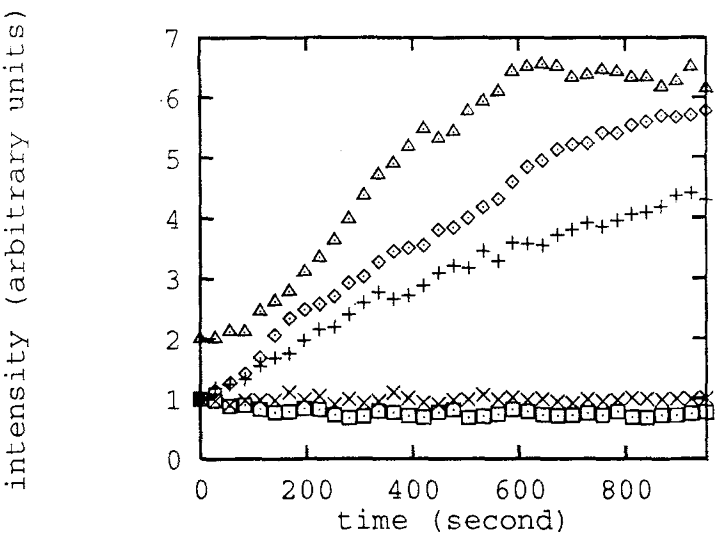

Figure 2. Time dependence of the intensity of the emission at 400 nm at ambient temperature in aqueous solutions of pH 12, saturated with N_2, and 10^{-3} M in tyrosyl units. (◇) $(Tyr)_1$, (+) $(Tyr)_2$, (□) $(Tyr)_3$, (×) $(Tyr)_6$, (△) $(Tyr)_{1000}$.

where $[A-A]$ denotes the concentration of bityrosine. The initial rate of the formation of $[A-A]$ is determined by $k_3(k_1[A]_0)^2$.

Figure 2 depicts the time dependence of the intensity of the emission at 400 nm for solutions saturated with diatomic nitrogen. The reaction is slow because of the low intensity of the incident irradiation, which was severely attenuated by passage of the incident beam through two double holographic gratings. Nevertheless, the data show similar rates for $(Tyr)_1$ and $(Tyr)_2$, lower rates for $(Tyr)_3$ and $(Tyr)_6$, and the fastest rate for the polymer. The initial rate is not a monotonic function of the degree of polymerization. As the degree of polymerization increases from 1 to 10^3, the initial rate of increase in the emission at 400 nm decreases, passes through a minimum, and then increases to a value larger than the result obtained with the free amino acid.

Another set of data, with faster development of the emission intensity at 400 nm, is presented in Figure 3. The solutions used in these experiments were saturated with N_2O instead of N_2. Nitrogen oxide can capture the aqueous electron which is produced in the initial reaction, Eq. (1). In basic solution, the result is the production of the OH• radical, which can further react with the tyrosine anion to produce more phenoxyl radicals (9,10). These additional phenoxyl radicals participate in the reaction in Eq. (2), thereby increasing the rate of increase in the intensity of the emission at 400 nm. This set of data, as with the set in Figure 2, shows that

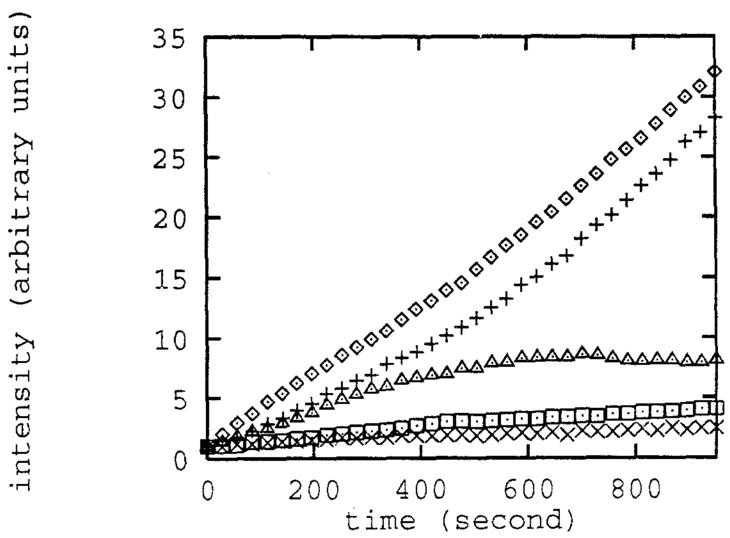

Figure 3. Time dependence of the intensity of the emission at 400 nm at ambient temperature in aqueous solutions of pH 12, saturated with N_2O, and 10^{-3} M in tyrosyl units. (◇) $(Tyr)_1$, (+) $(Tyr)_2$, (⊡) $(Tyr)_3$, (×) $(Tyr)_6$, (△) $(Tyr)_{1000}$.

the rate is lower for $(Tyr)_3$ and $(Tyr)_6$ than for the monomer, dimer, and polymer. The rate for the polymer is less sensitive to the presence of N_2O than are the rates for the monomer and dimer.

Discussion

Since all solutions have the same high concentration of tyrosyl units, nearly all of the incident light is absorbed by the solutions. Thus we assume there are negligible differences in the rates of the process depicted in Eq. (1). Then the factor which controls the relative rates of production of bityrosine in each set of experiments must be the rate of recombination, Eq. (2). For most reactions involving small molecules, k_3 is diffusion-controlled. If the recombination reaction in the oligomers is intermolecular, its rate should decrease with increasing degree of polymerization. Both the diffusion coefficient and the electrostatic repulsion of the negatively charged oligomers will increase as the degree of polymerization increases. Furthermore, the molar concentration of oligomer decreases as the degree of polymerization increases (it is the molar concentration of tyrosyl units, not the molar concentration of oligomer, that is held constant in the experiments). This rationale does not explain the large difference in rates between dimer and trimer, but it does rationalize the dominant trend observed with the oligomers.

If the same rational were extended to polymers with degrees of polymerization of 10^2 or 10^3, negligible rates should have been observed with those large molecules.

Clearly the data does not support that extension, because the rates measured for the polymers in both sets of experiments are signficantly larger than the rates observed with the trimer and hexamer. The polymers must have access to a mechanism for formation of bityrosine that is not accessible to a hexamer or smaller oligomer. The best candidate for that mechanism is the intramolecular formation of bityrosine by reaction of residues i and $i + j$. The very slow initial rate measured with $(\mathrm{Tyr})_6$ shows that $j > 5$. The fact that similar results were obtained with the two samples of the polymer, one having a degree of polymerization of 10^2, the other 10^3, argues that the typical values of j cannot be greater than 10^2. Hence we conclude that intramolecular formation of bityrosine is possible in poly(L-tyrosine) in dilute aqueous solution at pH 12, and that this intramolecular interaction involves tyrosine residues that are typically separated by $\sim 10^1$–10^2 units along the contour of the chain.

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