High Energy Radiation-Induced Aggregation of Plasma Proteins. Time Resolved Light Scattering Measurements

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Human immunoglobulin (IgG), bovine fibrinogen and bovine serum albumin were irradiated at room temperature with short pulses (50 ns to 2 µs) of 16 MeV electrons in 0.2 M $NaClO_4$ solution containing phosphate buffer (pH 7.5). In the presence of N_2O the intensity of light (LSI) scattered by the protein solutions increased after the pulse indicating aggregation. The latter process originates from the attack of protein molecules by OH radicals (aggregation is impeded by t-butanol and augmented by N_2O). Upon plotting the relative increase of LSI vs. log time (after the pulse) sigmoidally shaped curves were obtained.

From these findings and results of optical absorption measurements the following is inferred: Aggregation occurs as a consequence of chemical alterations of the protein molecules induced by the attack by OH radicals. The transient species formed during and a few µs after the pulse are reacting within a period of several ms. At the end of this period the formation of aggregates starts. It is concluded that the primary radiation chemical process consists (among others) in the generation of nucleation sites. The latter subsequently initiate aggregation processes.

Introduction

Kinetic and dynamic studies based on time resolved light scattering measurements concerning the change of size of macromolecules in solution have been carried out in our laboratory during the last years [1]. Those investigations were done by using the light scattering detection method in combination with the method of pulse radiolysis or flash photolysis. Predominantly synthetic polymers were used so far.

This paper reports first results obtained upon irradiating several proteins in dilute aqueous solution with short pulses (50 ns – 2 µs) of 16 MeV electrons. It has been reported [2, 3] that aggregation is the predominant effect observed upon irradiating proteins with ionizing radiation. Since the mechanism of aggregation is so far not understood, it was attempted to contribute to its elucidation by pulse radiolysis experiments in combination with time resolved light scattering measurements. Since the light scattering intensity (LSI) of a protein solution is expected to increase upon aggregation, it was expected that, this way, it should be possible to learn about the kinetics of aggregation.

As is described below, the three plasma proteins investigated exhibited phenomenologically an analogous behavior with respect to aggregation.

Experimental Part

(a) Materials

The proteins were lyophylized samples. They were used without further purification. *Human immunoglobuline* (IgG) was obtained from Miles. It consists of a mixture of the types IgG_1, IgG_2, IgG_3, and IgG_4. The average molecular weight amounts to about 1.5×10^5. *Bovine fibrinogen* (95% clottable) was also obtained from Miles. Its molecular weight is 3.4×10^5. Albumin (from bovine serum) was obtained from Boehringer (molecular weight: 6.8×10^4). For certain experiments t-butanol (Merck, p.a.) was used. $NaClO_4$ (p.a.), Na_2HPO_4 (p.a.) and KH_2PO_4 (p.a.) were obtained from Merck. For the sake of purification water was allowed to pass a set of Millipore cartridges (ion exchange, super carbon and filtration cartridges).

(b) Preparation and Irradiation of Samples

Protein samples were dissolved several hours prior to irradiation in 10^{-2} M phosphate buffer solution (pH 7.5) containing $NaClO_3$ (0.2 mol/l). The solutions were saturated with either Ar or N_2O. The latter converts hydrated electrons to OH radicals

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$(e_{aq}^- + N_2O + H_2O \rightarrow OH\cdot + OH^- + N_2)$. Some experiments were carried out with air-saturated solutions.

For the irradiations a flow system was used. The sample cell (quartz) was placed directly in front of the exit window of a L-band linear accelerator (Vickers). The samples were irradiated with single pulses of 16 MeV electrons. The pulse duration was varied between 50 ns and 2 μs. The absorbed dose per pulse amounted from 4 to 150 krad. It was determined with the aid of the Fricke dosimeter.

For the light scattering measurements an argon-ion laser (Spectra Physics, Model 165-00, 1.2 W at $\lambda = 514.5$ nm) was used. Details concerning the pulse radiolysis facility used (including the LS and the optical absorption detection system) have been described elsewhere [1, 4, 5].
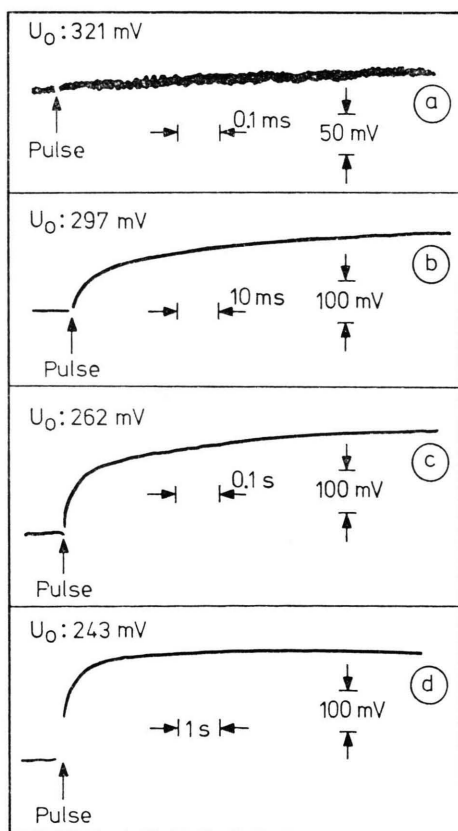
Results

(a) Light Scattering Measurements

The three proteins exhibited an analogous behavior upon irradiation with an electron pulse in N_2O-saturated solution. Typical oscilloscope traces obtained with a IgG solution of 2 g/l are depicted in Fig. 1, where one can see that the LSI increases steadily after the pulse. By using a logarithmic time scale sigmoidal curves were obtained demonstrating that the saturation value is reached in the range of seconds. Typical curves obtained with IgG and albumin are shown in Fig. 2 (a) and (b).

The extent of the LSI increase is increasing steadily with the absorbed dose per pulse as is shown in Fig. 3 for the three proteins. Upon prolonged irradiation aggregates precipitated in all cases. It appears
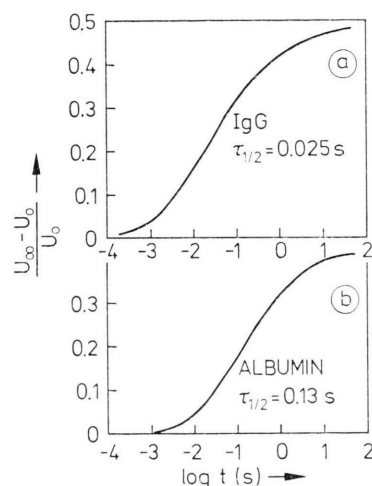

Fig. 1. Irradiation of IgG (2 g/l) in N_2O-saturated 10 mM phosphate buffer solution (pH 7.5) at room temperature. [NaClO₄]: 0.2 M. Absorbed dose per pulse: 90 krad. Typical oscilloscope traces demonstrating the increase of LSI after the pulse as a function of time. The time scale increases from (a) to (d) as indicated in the graph.

Fig. 2. Semilogarithmic plot demonstrating the increase of the LSI after the pulse as a function of time. N_2O-saturated solutions (10 mM phosphate buffer, pH 7.5, [NaClO₄]: 0.2 mol/l) of (a) IgG (2 g/l, abs. dose per pulse 21 krad) and (b) albumin (2 g/l, abs. dose per pulse: 42.5 krad).
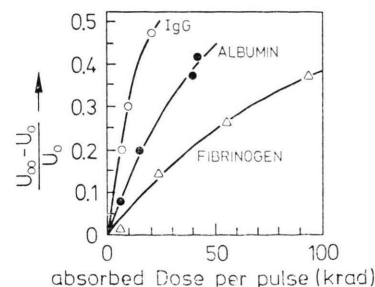

Fig. 3. The extent of LSI increase as a function of absorbed dose per pulse for IgG, albumin and fibrogen in N_2O-saturated solution. Protein concentration 2 g/l.

Table 1. Irradiation of immuno-globulin. Total extent of LSI increase at constant absorbed dose per pulse (28 krad). IgG (2 g/l) in 10^{-2} M phosphate buffer (pH 7.5). [$NaClO_4$]: 0.2 M.

Condition [a]	$\Delta U/U_0$ [b]
N_2O	0.30
Ar	0.15
Ar, t-butanol $(1.5 \times 10^{-1}$ M)	0
air	0

[a] Solutions were saturated with the gas indicated.

[b] $\Delta U = U_\infty - U_0$. U_0 and U_∞ designate the signal voltages (proportional to the LSI) before and a long time after irradiation.

Fig. 4. Absorption spectrum measured at the end of a 2 µs pulse. Fibrinogen (3 g/l) in N_2O saturated 10 mM phosphate buffer solution, pH 7.5 [$NaClO_4$]: 0.2 M. Absorbed dose per pulse: 12 krad.

that the aggregation originates with the attack of the protein molecules by OH radicals. This is inferred from the following findings: If t-butanol (a scavenger for OH radicals) was contained in Ar-saturated solution at a sufficiently high concentration the formation of aggregates was suppressed. Typical results obtained with IgG are presented in Table 1. It is seen that the total extent of the LSI increase observed in N_2O saturated solution is augmented relative to that of the Ar-saturated solution, indicating that hydrated electrons are not becoming operative with respect to aggregate formation. (Due to the conversion of e_{aq}^- to OH radicals, it is expected on the basis of the radiation chemical yields that the extent of the observed effect roughly doubles [6].)

In the presence of oxygen (i. e. in air-saturated solutions) the formation of aggregates was prohibited at relatively low doses per pulse (<50 krad). At higher doses aggregation was observed. However, the total extent of LSI increase was much smaller than in Ar-saturated solution.

(b) Optical Absorption Measurements

These measurements were of importance with respect to two points: (i) Chemical reactions induced by the electron pulse might be detectable via the optical absorption of transients. The question, therefore, arose as to whether the rates of formation and/or decay of the transient absorption are correlated with the rate of aggregate formation. (ii) The LSI measurements were carried out at $\lambda =$
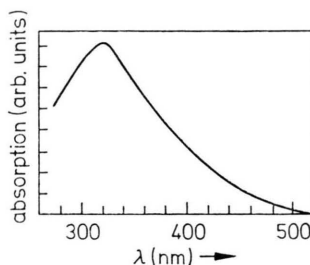
514 nm. Optical absorption changes in the scattering solution at this wavelength are reflected by the LSI detection system and might, therefore, lead to artifacts.

In all cases a transient absorption was formed during the pulse emerging at about 530 nm and increasing with decreasing wavelength in the observed range down to about 300 nm. Absorption maxima between 320 and 350 nm were observed. A typical transient spectrum recorded at the end of a 2 µs pulse in the case of fibrinogen is shown in Figure 4. At wavelengths below 400 nm the absorption decayed according to two modes corresponding to halflife times of 0.1 to 1 ms and 2 to 5 ms, respectively. At higher wavelength only the slow mode of decay was observed. At 514 nm the absorption amounted only to a few per cent. The half-life time is significantly shorter (by one or two orders of magnitude) than the half-life times found for the formation of aggregates. Thus, the decay of the transient absorption certainly does not overlap with the strong increase of the LSI. Overlapping occurs, however, in the time range where the formation of aggregates starts, i. e. during the "nucleation" period.

Conclusions

The results obtained from the optical absorption measurements show: (a) During the period of significant LSI increase a transient at 514 nm does not influence the LSI measurements (b) Intermediates formed during and after the pulse are decaying at an early stage relative to the aggregation process. The light scattering measurements indicate the following: The curves depicting the LSI increase after the pulse are of sigmoidal shape. This strongly suggests that we are dealing here with aggregation processes proceeding analogously to those induced

by other means [7]. It appears that the slow initiation period observed corresponds to the "nucleation" period usually found in the aggregation of proteins. The initiation period overlaps with the decay of the transient species. Therefore, it is concluded that aggregation occurs as a consequence of chemical alterations of the protein molecules induced by the attack of the latter by OH radicals

A transient absorption indicating lifetimes of protein radicals extending to the period of pronounced aggregation was not detectable. Therefore, the possibility can be excluded that the aggregation is due to the combination of protein radicals.

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