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Received September 4, 1963.

The Decay of Radiation-Induced Free Radicals in Egg Albumin at Different Water Contents

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Radiation denaturation of proteins in the solid state, measured for example as enzyme inactivation, is being more and more investigated. The water content effect on the radiation damage in seeds [1,2] and starch [2,3] has been well established. The radiation damage in enzymes also seems to be influenced by the water content.[4] For these reasons it was decided to make an investigation of the decay of radiation-induced free radicals in a protein at various water contents and to compare the data with those obtained from studies on whole seeds.[2]

In this investigation egg albumin was used, which was prepared by P. Perlmann according to the method of Kekwick and Cannan.[5,6] The egg albumin, originally containing 19 % water, was equilibrated to different water contents by means of prolonged storage in atmospheres of controlled humidity.[1] The water content was calculated from the loss of weight during desiccation, and was also checked by drying to constant weight at 105°C. The samples were transferred to Pyrex tubes (3 mm inner diameter) and were evacuated three times for 5 min. Between each evacuation, the samples were washed with oxygen-free nitrogen (from British Oxygen) and the tubes were finally filled with nitrogen. The samples were irradiated with ^{60}Co γ-rays during 1 h, and received a dose of 0.43 Mrad. They were measured by electron spin resonance (ESR) within 2 h after irradiation and the decay of radicals was followed an appropriate length of time. Technical data of the ESR equipment and absolute measurements of radicals are described elsewhere.[7] Some samples were opened after the first measurements, and the decay in air was followed. Irradiation and ESR measurements were made at room temperature.

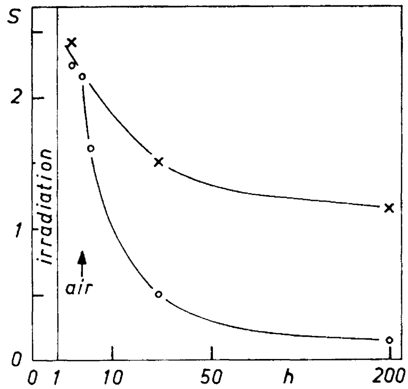

Fig. 1. Decay curves for radicals in egg albumin containing 0.6 % water; upper curve in nitrogen, lower curve in air from the time indicated. S is in units of radicals $\times$ g^{-1} $\times$ 10^{17}.

The ESR spectrum and its change with time agree at least qualitatively with the results of Polatova *et al.*[8] Two typical decay curves are shown in Fig. 1. No simple rate equations can be fitted to the complete curves. The best attempted approximation seems to be a second order reaction if a semi constant level is introduced, as in decay reactions for radicals in seeds.[2]

An extrapolation of the decay curve to time zero, *i.e.* the end of the irradiation period, and a correction of this extrapolated value for the decay during the irradiation, using the previously mentioned second order reaction approximation, gives the total amount of radicals, S_0, which has been induced. This value, of course, does not include such types of radiation-induced radicals which decay so rapidly that they have already vanished completely at the first measurement. In Fig. 2, where S_0 is plotted *versus* water content, it can be seen that there is a strong water content

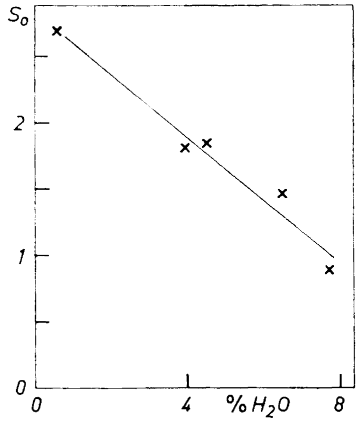

Fig. 2. Initial concentration, S_0, of radicals (*cf.* text) in egg albumin *versus* water content. The concentrations are calculated per dry weight of protein. S_0 is in units of radicals $\times$ g^{-1} $\times$ 10^{17}.

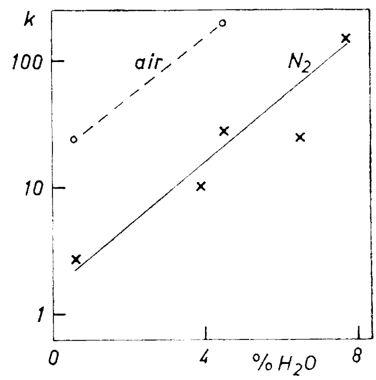

Fig. 3. Decay of radicals in egg albumin as a function of water content, where k is the velocity constant of the second order decay; k is in units of radicals^{-1} $\times$ g $\times$ h^{-1} 10^{-19}.

influence on the production of radicals, which decay slowly enough to permit measurement. (A small part of the slope of the line in Fig. 2 is due to an increase of the dielectric losses with increasing water content.[2]) Assuming that the total yield of radicals is independent of water content, which has been found for seeds,[2] one finds that a great part of the induced radicals at higher water content decay too fast to permit measurement in these experiments. The G-value, *i.e.* the number of free radicals per 100 eV, for the driest sample is 0.9, being within the range of values found for other proteins.[9] The velocity constant, k, of the attempted second order decay is a measure of the decay rate. In Fig. 3 k is plotted, in log scale, *versus* water content. It can be seen that k varies with a factor of two with a change of water content of 1 %, and with a factor of about ten when the nitrogen atmosphere is replaced by air.

The above given data show that the production of radicals in egg albumin, and their decay, are strongly dependent on the water content. Though it is impossible to apply these results in detail to other proteins, it is our opinion that the data can be of value when radiation damage on solid proteins is studied by chemical methods.

Acknowledgement. The author wishes to express his special thanks to Dr. A. Ehrenberg and Professor L. Ehrenberg for suggestions ans stimulating discussions.

The present investigation was aided by grants from *Knut och Alice Wallenbergs Stiftelse, Riksföreningen mot Cancer, Statens Råd för Atomforskning* and Public Health Service grant AM-05895 from the *National Institute of Arthritis and Metabolic Diseases.*

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