

Kinetics of thermal decay of antigen E from short ragweed pollen*

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The rate of thermal decay of crude and purified preparations of antigen E from pollen of the short ragweed was determined at 4, 24 and 35 °C in the absence of preservative and in the presence of phenol, parabens or thimerosal. Arrhenius plots for the crude antigen preparations indicated the participation of two or more different processes, but the plot for the purified antigen illustrated a single process with an energy of activation of 16.4 kcal/mol. From the theory of absolute reaction rates, ΔH was calculated to be 14.9 ± 1.0 kcal/mol, and ΔS to be -13.3 ± 3.5 cal/deg/mol.

INTRODUCTION

In a recent report of the effect of preservatives upon the antigenic stability of short ragweed pollen extracts, Baer & Hooton (1974) presented data for both crude and purified extracts maintained without preservatives at 4, 24 and 35 °C for periods up to 160 days. Parallel experiments were conducted with glycerol (50%), phenol (0.1-0.8%), parabens (0.15% methyl; 0.05% propyl) or thimerosal (1 : 10 000). Glycerol was qualitatively different from the other agents tested in that it protected both crude and purified extracts from any significant loss of antigenic activity, even after 160 days at 35 °C. The other agents had little or no significant effect upon thermal stability of the antigen.

The present report presents an analysis of the kinetic parameters for the thermal decay reaction over a period of 320 days for all of the extracts except those preserved with glycerol.

MATERIALS AND METHODS

As previously reported (Baer & Hooton, 1974), short ragweed pollen (1964 crop year) was defatted in a Soxhlet apparatus using petroleum ether of boiling range 30-60 °C. The

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defatting proceeded until the extracting fluid appeared colorless, approximately 6 h. The pollen was permitted to remain at room temperature overnight to allow all petroleum ether to evaporate. The pollen (in a ratio of 1 part pollen to 10 parts of extracting fluid) was extracted either with Coca's solution (Coca, 1922) without phenol, or with 50% glycerol. Extraction took place at 24 °C with constant stirring for 24 h. The extracts were centrifuged and passed through a membrane filter (45 µm porosity) for sterilization. To portions of those extracts containing no glycerol, solutions were added (1 part phenol in Coca's solution to 10 parts of extract) to give phenol concentrations of 0.1, 0.2, 0.4 and 0.8%. To other portions of the extracts in Coca's solutions were added parabens (final concentration of 0.15% methyl and 0.95% propyl) and thimerosal (final concentration 0.01%).

Similar solutions were prepared in which the short ragweed pollen extract was first diluted 1 : 8 in Coca's solution; in addition, solutions of antigen E were prepared with the same preservatives. Because of the small quantities of antigen E available, only one set of concentrations could be used; the 1 : 8 dilution of short ragweed pollen extract had an antigen E concentration similar to the pure antigen E solutions.

Portions of each of the solutions in sterile screw-capped vials were stored at 4, 24 or 35 °C for up to 320 days.

The entire study, starting with pollen, was repeated once in an identical manner, the results being the mean of both experiments.

Antigen E, a protein antigen of molecular weight 37 800 (King, Norman & Connell, 1964) is considered to be the most important allergen extracted from short ragweed pollen. The material used in this study was fraction IV-c with $\epsilon_{280}^{1\%} = 11.8$. The concentration of antigen E in the extracts and solutions was determined by radial immunodiffusion (Williams & Chase, 1970). A standard curve was prepared using the US Reference antigen E, lot 1, and sheep anti-antigen E serum, lot 1. The coefficient of variation for individual assays was approximately 11.4%.

RESULTS

First-order velocity constants (k) for each preparation were calculated for each temperature by a least-squares fitting of the function

$$\ln C/C_0 = -kt \quad (1)$$

in which C is the observed antigen concentration at time t and C_0 the initial concentration. For presentation in Tables 1-3, the velocity constants were converted to half-lives ($t_{1/2}$) as

$$t_{1/2} = 0.693/k. \quad (2)$$

The significance of differences in half-lives among the groupings of interest in Tables 1-3 was determined by Kruskal-Wallis one-way analysis by ranks (Siegel, 1956) with the statistic H defined as

$$H = \frac{12}{N(N+1)} \sum_{j=1}^k \frac{R_j^2}{n_j} - 3(N+1) \quad (3)$$

in which N is the total number of half-lives, k is the number of groupings compared, n_j is the number of samples in the j th grouping and R_j is the sum of ranks in the j th grouping. Degrees of freedom are defined as d.f. = $k-1$, and the probability associated with H is obtained from tabulated critical values of chi-square.

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THERMAL DECAY OF ANTIGEN E

TABLE 1. Half-lives of crude ragweed pollen antigen in the presence of various antibacterial agents (initial antigen concentration, 360 µg/ml)

Antibacterial agent	Half-life (days)		
	4 °C	24 °C	35 °C
None	> 320	> 320	5.0
Phenol, 0.1%	> 320	94	4.9
0.2%	> 320	84	4.9
0.4%	> 320	78	4.8
0.8%	> 320	59	4.8
Parabens	> 320	83	4.8
Thimerosal	> 320	76	4.7
Glycerol	> 320	> 320	> 320

From Tables 1 and 2 it may be seen that the crude antigen, at either of the initial concentrations, was relatively stable at 4 °C, regardless of the preservative used, and apparent variations in half-life are largely attributable to experimental error in the assays. The purified antigen was less stable at 4 °C than either of the crude preparations ($H = 14.3$, $P < 0.001$) and the purified antigen containing 0.1% or more phenol was significantly less stable at 4 °C ($H = 4.5$, $P < 0.01$) than the preparations containing no preservative, parabens or thimerosal.

TABLE 2. Half-lives of crude ragweed pollen antigen in the presence of various antibacterial agents (initial antigen concentration, 40 µg/ml)

Antibacterial agent	Half-life (days)		
	4 °C	24 °C	35 °C
None	> 320	286	8.4
Phenol, 0.1%	> 320	154	9.0
0.2%	> 320	153	9.4
0.4%	> 320	134	9.6
0.8%	> 320	123	9.7
Parabens	> 320	221	9.0
Thimerosal	> 320	114	10.8
Glycerol	> 320	> 320	> 320

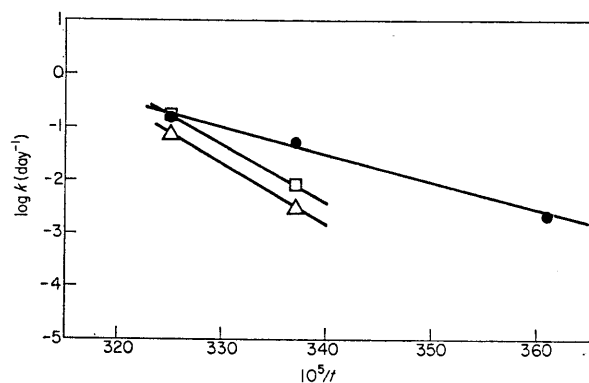
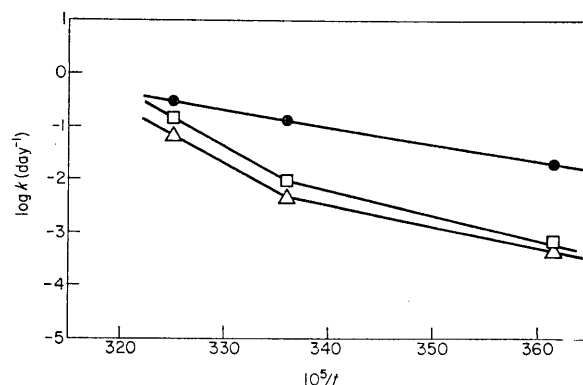
At 24 and 35 °C the deleterious effect of the preservatives was more apparent. Interestingly, the crude antigen in dilute concentration (Table 2) was more stable ($H = 8.3$, $P < 0.01$) in the presence of preservatives than the crude antigen in concentrated form (Table 1).

With the purified antigen (Table 3) the adverse effect of preservatives was proportionally greater at lower temperatures and, at 35 °C, parabens, thimerosal and phenol at 0.1% concentration had no significant effect upon thermal stability ($H = 0.18$, $P < 0.50$). Taking the data as a group, one finds that phenol at higher concentrations did not contribute significantly ($H = 3.5$, $P < 0.05$) to the thermal decay rate of purified antigen at this temperature.

The effect of temperature upon the velocity constants for the thermal decay reaction is shown in the form of Arrhenius plots in Figs 1-4. As a common feature of all these

TABLE 3. Half-lives of purified ragweed pollen antigen in the presence of various antibacterial agents (initial antigen concentration, 45 $\mu\text{g/ml}$)

Antibacterial agent	Half-life (days)		
	4 °C	24 °C	35 °C
None	235	14.5	5.3
Phenol, 0.1%	67	7.8	6.0
0.2%	24	6.1	1.8
0.4%	88	10.7	3.9
0.8%	22	1.8	0.9
Parabens	318	13.4	5.3
Thimerosal	219	13.9	4.5
Glycerol	> 320	> 320	195

Fig. 1. Arrhenius plot for thermal decay of ragweed pollen antigen E in the absence of preservative. Purified antigen (●—●); crude antigen at an initial concentration of 360 $\mu\text{g/ml}$ (□—□); crude antigen at an initial concentration of 40 $\mu\text{g/ml}$ (△—△).Fig. 2. Arrhenius plot of pooled data for thermal decay of ragweed pollen antigen E in the presence of 0.1, 0.2, 0.4 or 0.8% phenol. Purified antigen (●—●); crude antigen at an initial concentration of 360 $\mu\text{g/ml}$ (□—□); crude antigen at an initial concentration of 40 $\mu\text{g/ml}$ (△—△).

plots, the data for the purified antigen appear to represent a single, classical type of temperature dependence, with a calculated mean value of 16.4 kcal/mol for the Arrhenius energy of activation. By the method of absolute reaction rates (Eyring, 1935) ΔH for the reaction is estimated to be 14.9 ± 1.0 kcal/mol, and ΔS to be -13.3 ± 3.5 cal/deg/mol.

THERMAL DECAY OF ANTIGEN E

Thermal decay of the crude antigens proceeds in a more complicated way, with evidence for participation of at least two different reactions, but the data are insufficient to suggest a definitive model for the process.

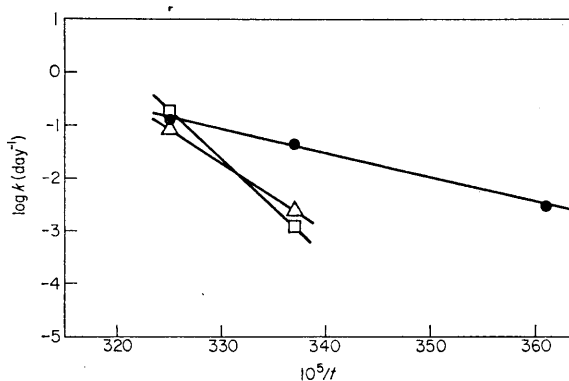


Fig. 3. Arrhenius plot for thermal decay of ragweed pollen antigen E in the presence of 0.15% methylparabens and 0.05% propylparabens. Purified antigen—($\bullet$ — $\bullet$); crude antigen at an initial concentration of 360 $\mu\text{g/ml}$ ($\square$ — $\square$); crude antigen at an initial concentration of 40 $\mu\text{g/ml}$ ($\triangle$ — $\triangle$).

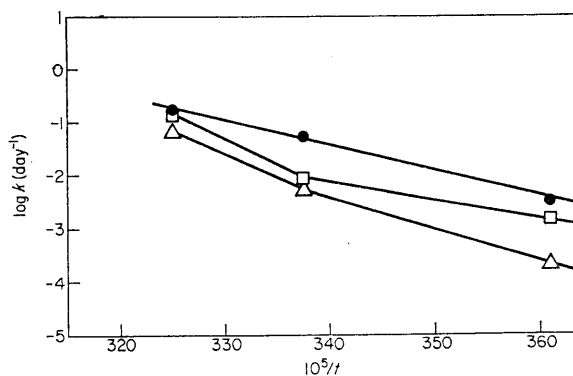


Fig. 4. Arrhenius plot for thermal decay of ragweed pollen antigen E in the presence of thimerosal (Merthiolate) 1:10 000. Purified antigen ($\bullet$ — $\bullet$); crude antigen at an initial concentration of 360 $\mu\text{g/ml}$ ($\square$ — $\square$); crude antigen at an initial concentration of 40 $\mu\text{g/ml}$ ($\triangle$ — $\triangle$).

DISCUSSION

The stability of biological products has usually been approached pragmatically, with little concern about theoretical aspects. As a result, most of the data have been obtained at temperatures too low for accurate rate determination. In the recent report by Center, Schuller & Zeleznick (1974), for example, the half-life of ragweed antigen E in commercially prepared extracts was found to be about 8 months at 4 °C and 2 months at 24 °C, but the experiments were not designed to yield estimates of the fundamental thermokinetic parameters. Peddecord, Northey & Goodman (1976) reported data on deterioration of antigen E, leading to a calculated half-life of about one year at 5 °C, but in the absence of data at other temperatures no value of ΔH or ΔS was calculable.

While the mechanisms of thermal decay of biological products are manifold and for the most part undetermined (in the sense that chemical reactions cannot be specified), systematic collection and reporting of ΔH and ΔS values may be of ultimate value to investigators who wish to generalize about reaction mechanisms or are searching for a broad frame of reference for their own observed data. Stearn (1949) discusses many of the ways in which kinetic data may serve to elucidate structures and mechanisms. Woese (1960) uses data for a variety of biological substances in comparison with findings related to the thermal decay of animal viruses. We hope that subsequent investigators will elect to report stability data on allergenic extracts and other biological products in thermodynamic terms, so that a useful body of knowledge will eventually accumulate.

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