Complement: Increased Efficiency of the Second Component after Treatment with Iodoacetamide

Abstract. *The apparent activity of the second component of human complement was enhanced by treatment of the purified protein with iodoacetamide. By contrast, treatment with iodoacetic acid or* p-*chloromercuribenzoate led to inactivation. Treatment with iodoacetamide prevented the effect of* p-*chloromercuribenzoate and vice versa. Enhanced activity was partly due to increased stability of the otherwise labile intermediate complex consisting of erythrocytes, antibody, and the first, second, and fourth components of complement.*

The course of immune hemolysis by complement is greatly influenced by the marked lability of one of the intermediate complexes produced during this reaction. The complex consists of erythrocyte (E), antibody (A), the first (C′1), second (C′2), and fourth (C′4) components of complement, and is referred to as EAC′1a,4,2a (*1*). Mayer and his associates (*2*) have shown that EAC′1a,4,2a tends to revert to the EAC′1a,4 state, the half-life of the labile complex being 8 minutes at 37°C. Using an immunologic technique, Mayer (*3*) also demonstrated that decay of EAC′1a,4,2a corresponds to dissociation of C′2 from the complex.

During the process of isolation of C′2 from human serum, increasing loss of C′2 activity was encountered. Since ethylenediaminetetraacetic acid (EDTA) prevented this inactivation, the activity may have been dependent upon a free sulfhydryl group in the C′2 molecule. This was particularly likely in that C′2 is inactivated by *p*-hydroxymercuribenzoate (*4*). Exploring further the effect of sulfhydryl blocking reagents on C′2 activity, the observation was made that treatment with iodoacetamide resulted in marked enhancement of C′2 activity.

The C′2 was isolated from human serum in highly purified form (*5*). Treatment with sulfhydryl reagents was carried out at a protein concentration of 100 to 200 μg/ml. The final concentration of sulfhydryl reagent in the protein solution was 0.025 or 0.05*M* for iodoacetamide, 0.05*M* for iodoacetic acid, and 0.001*M* for *p*-chloromercuribenzoate (*p*-CMB). Treatment was performed in phosphate buffer, *p*H 6; ionic strength = 0.1 (found to be optimal for iodoacetamide); and usually for 30 minutes at room temperature, although the effects of the reagents were almost instantaneous. For the determination of hemolytic activity, samples of treated C′2 were diluted at least 100-fold. The concentration of the sulfhydryl reagents was thus rendered ineffective in the hemolytic assay system, as indicated by control experiments. Furthermore, dialysis of treated C′2 prior to the hemolytic assay yielded similar results.

The effect of the three sulfhydryl reagents on C′2 was estimated with a serum reagent lacking C′2 (*6*, p. 162). This method revealed a reduction of C′2 activity by iodoacetic acid and *p*-CMB to approximately 10 and 20 percent, respectively. Iodoacetamide, however, produced a 600 to 1000 percent increase in activity.

To obtain more accurate data on the extent of the iodoacetamide effect, formation and decay of EAC′1a,4,2a, with iodoacetamide-treated and untreated C′2, were determined as a function of time. For these experiments, EAC′1a,4 cells were prepared with sheep erythrocytes, rabbit antibody to sheep erythrocytes, the subcomponents of human C′1 (C′1q, C′1r, C′1s) (*7*), and human isolated C′4 (*8*). Formation of EAC′1a,4,2a was allowed to proceed for various periods of time as indicated in Fig. 1. The complex was then quantitated by determining the degree of hemolysis after incubation with EDTA-containing human serum for 60 minutes at 32°C. Figure 1 demonstrates that the half-life of EAC′1a,4,2a prepared with iodoacetamide-treated C′2 was more than ten times greater (approximately 150 minutes) than that of EAC′1a,4,2a prepared with untreated C′2 (13 minutes). In addition, the extent of EAC′1a,4,2a formation was more than seven-fold greater with the iodoacetamide-treated C′2 than with the control. This difference is too large to be explained solely by the increase in half-life of EAC′1a,4,2a in view of Mayer's observations with guinea pig C′2 (*6*, p. 203). The theoretical value for EAC′1a,4,2a formation determined by Mayer at the time of maximal formation and corrected for decay of the complex was not more than 50-percent higher than the observed value. If the results with guinea pig C′2 are applicable to human C′2, the data illustrated in Fig. 1 indicate that treatment of human C′2 with iodoacetamide resulted not only in greater stability of the EAC′1a,4,2a complex, but also in an apparent increase in its formation. The exact nature of the effect of iodoacetamide on human C′2 remains to be determined.

The finding that two different sulfhydryl reagents had opposite effects on

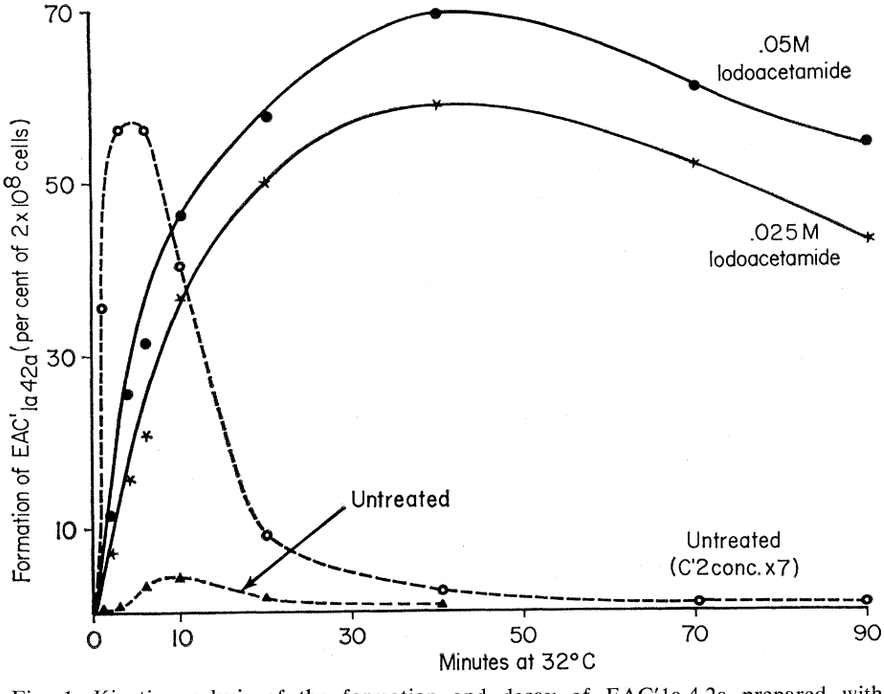

Fig. 1. Kinetic analysis of the formation and decay of EAC′1a,4,2a prepared with iodoacetamide-treated and untreated C′2. Treatment with iodoacetamide was carried out at a final concentration of 0.05*M* and 0.025*M*. Untreated C′2 was tested at the same concentration as the iodoacetamide-treated C′2 and at seven times this concentration.

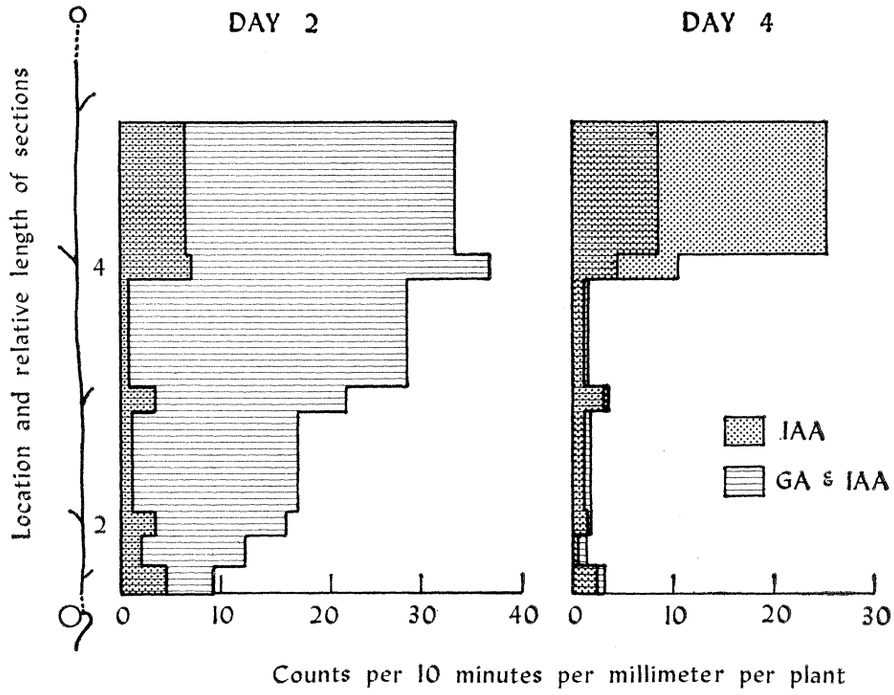

Fig. 1. Average counts obtained from ether extracts of successive nodes and internodes of pea stems, as shown on the left. Five plants were used per treatment. The stem had been decapitated about midway up the 6th internode. For isotope counting the stem just below the paste was discarded (dashed line). The remainder of internode 6 plus node 5 (not graphed) gave counts of 399 and 505 after treatments with IAA-C^{14} for 2 and 4 days, respectively. Corresponding portions from plants treated with gibberellic acid plus IAA-C^{14} gave 322 and 415 counts, respectively.

regions of the stem remote from the site of application (Fig. 2). In the R_F zone typical of IAA was found 33 percent of the radioactivity from chromatographed extracts of the bottom half of pea stems which had IAA-C^{14} plus gibberellic acid substituted for their apical shoot tip. Only 10 percent of the smaller amount of total radioactivity was in the "IAA" zone when IAA-C^{14} alone was substituted for the apical shoot tip.

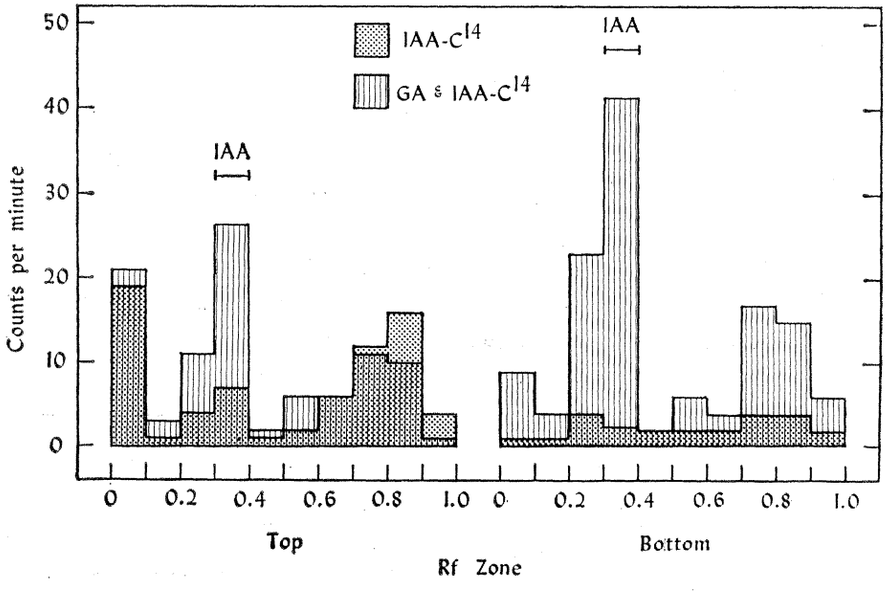

Fig. 2. Average counts from chromatograms of ether extracts of plants treated for 2 days. The solvent was isopropanol-ammonia-water (8 : 1 : 1). The dried zones were counted directly in scintillation fluid (*8*). The calibration IAA-C^{14} was run at the same time in a separate chromatogram tube. "Bottom" designates extracts from the bottom portion of the stem (nodes 3 and below). "Top" designates extracts of node 4 plus internodes 4 and 5 ($N = 15$).

The time-course of this gibberellic acid effect is closely correlated with the time-course of side-shoot inhibition (*5*).

There is no reason to think that gibberellic acid is inhibiting side shoots indirectly by stimulating elongation of the main shoot. There were no significant differences in total lengths of the treated main shoots on either day 2 or day 4 (Table 1). Nor was there any significant difference in the average stump length of internode 6 at the start of the experiment. [The plants were decapitated about half-way up the 6th internode. Stumps used for IAA treatment averaged 14.4 mm ± 0.6 ($N = 15$); those treated with gibberellic acid plus IAA treatment averaged 15.3 ± 0.6 ($N = 14$).]

This work provides evidence that both gibberellins and IAA are normally involved in maintaining apical dominance in pea plants, and that gibberellic acid acts by increasing the concentrations of IAA functional at a distance from the site of production.

The physiological-biochemical mechanism by which gibberellic acid exerts this effect remains to be investigated. One of the most attractive possibilities is that gibberellic acid increases the transport of IAA. In addition to the earlier reports of gibberellic acid decreasing the growth of side shoots (*7*), a number of otherwise puzzling observations can be explained if gibberellic acid increases auxin transport in other situations. Among these are: (i) the frequent need for adding auxin before the growth effects of gibberellic acid become apparent (*7*); (ii) the stimulation by gibberellic acid of xylem differentiation in the roots of intact bean seedlings (*9*), whereas auxin is the normal limiting factor for the differentiation of xylem cells in *Coleus* stems and stimulates the process in pea roots, fern leaves, and lilac callus (*3*, *10*); (iii) the increase in the amount of endogenous "diffusible" auxin collected after gibberellic acid application (*11*). Although Kuraishi and Muir (*11*) suggested that gibberellic acid may directly increase the *formation* of auxin, it is well to remember that the collection of diffusible auxin requires transport. Even the mysterious fact that gibberellic acid added to an intact plant causes growth, whereas IAA does not (*7*), seems explicable as an effect of gibberellic acid on auxin transport, since quantitative evidence exists that