A Small Angle X-Ray Scattering Study of Sodium Humate Solutions

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The first small angle X-ray scattering study of humates was made by Wershaw *et al.*[1] The Guinier plots obtained showed two straight lines, and the different possibilities to explain this experimental fact were discussed: "There may be particles of different sizes or particles of uniform size consisting of different zones of different electron density." Wershaw is continuing his studies in order to distinguish between the two possibilities, and this short communication will only contribute some additional facts with no anticipation of the final explanation.

Experimental. The *chernozem* humate investigated at pH 7 has been studied earlier [2] (and was then numbered 1 N2 ESB). The same fraction has also been hydrolyzed by boiling HCl (as described earlier [2]) and afterwards dissolved in NaOH to pH 7. (The concentrations of the solutions studied are not accurately known but are in the order of 0.5—1 g humic acid/l solution.) The small angle X-ray scattering was studied with a Kratky camera using CuK radiation (with a pulse-height analyzer attached to the output of a proportional counter). The X-ray beam was collimated with an entrance slit of 175 μ. The glass capillaries used were 1 mm thick.

Results. The results of Wershaw were confirmed in so far that more than one straight line was obtained in the Guinier plot for the *chernozem* humate (Fig. 1). (In fact even more than two lines were observed in this case as well as for some other humates which were preliminary studied.) The hydrolyzed sample on the other hand (Fig. 2) gave only one straight line in the

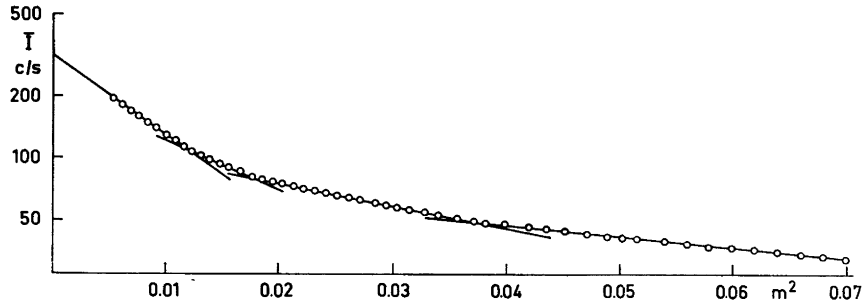

Fig. 1. The log of scattered intensity, $\overline{I}$, measured in counts/sec is plotted against m^2, the distance from the primary beam to the detector, measured in cm and then squared (Guinier plot). Only a part of the measured values is reproduced. *Chernozem* humate solution.

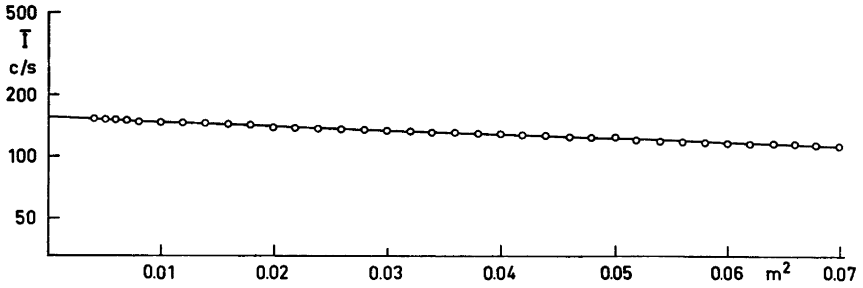

Fig. 2. A Guinier plot as in Fig. 1. Hydrolyzed *chernozem* humate solution.

same range of angles, corresponding to a radius of gyration of 18—19 Å. It is obvious that the acid hydrolysis had degraded the humate considerably (as also indicated by gel filtration in an earlier study [2]). The detailed picture of this degradation depends on the explanation finally given to the occurrence of more than one straight line in the Guinier plot of the original humate. The hydrolyzed humate will be studied further with small angle X-ray scattering.

1. Wershaw, R. L., Burcar, B. P., Sutula, C. L. and Wiginton, B. J. *Science* **157** (1967) 1429.
2. Lindqvist, I. *Lantbrukshögsk. Ann.* **34** (1968) 377.

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The Transient State Kinetics of Horse Liver Alcohol Dehydrogenase*

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The rapid mixing technique has made it possible to study the "pre-steady" state kinetics of enzyme reactions. When Bernard *et al.*[1] reported two distinct rate steps for the conversion of aromatic aldehydes into aromatic alcohols, we presumed that the initial rapid step at the "pre-steady" state is due to the rapid transformation of "ERald" to "EOalc" followed by the rapid dissociation of EOalc to EO+alc as shown in the following.**

$$\mathrm{EO+alc} \underset{k_{-2}'}{\overset{k_2'}{\rightleftharpoons}} \mathrm{EOalc}$$

$$\mathrm{EOalc} \underset{k_3}{\overset{k_{-3}}{\rightleftharpoons}} \mathrm{H^+ + ERald}$$

$$\mathrm{ERald} \underset{k_2}{\overset{k_{-2}}{\rightleftharpoons}} \mathrm{ER + ald}$$

Therefore, experiments were carried out to determine the rate constants of the individual steps using our "stopped-flow" spectrofluorimeter.

The solutions of E+R or E+O (E≪R or E≪O) were rapidly mixed with varying concentrations of different substrates in glycine-NaOH buffer, pH 9.0. A rapid appearance of the strong fluorescence of bound NADH is followed by a relatively slow appearance of the much weaker fluorescence of free NADH in the case of EO+ethanol or benzylalcohol (ER→E+R). A very rapid decrease of the ER fluorescence is followed by a slow decrease of the free R fluorescence in the case of the aldehydes being the substrates. ER and ERald seem to have the same fluorescence intensity as judged from "stopped flow" experiments in which ER was mixed with increasing concentrations of aldehydes. The total deflections after mixing kept reasonably constant until so high concentrations of aldehyde were reached that appreciable hydride transfer occurred during the "dead time" before observation.

A plot of the reciprocal of the initial rates of the rapid appearance of ER and ERald fluorescence against the reciprocal of the ethanol or benzylalcohol concentrations is shown in Fig. 1. The rate constants from the maximum velocities are calculated to be 100 sec^{-1} and 6.4 sec^{-1} for ethanol and benzylalcohol, respectively. The disappearance of the bound NADH fluorescence caused by acetaldehyde or benzaldehyde was too fast to be followed accurately. However, the approximate rate constants were estimated to be 300—500 sec^{-1} for both cases. These rate constants should correspond to k_3 or k_{-3} which represent the rate of the hydride transfer. k_2 or k_2' can also be obtained from the slope of the plot shown in Fig. 1. From determinations of the dissociation constants of ERald or EOalc from the kinetics of the product inhibition,[2] it is possible to calculate k_{-2} or k_{-2}'. The data of the rate constants were

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** Abbreviations: E, LADH; R, NADH; O, NAD^+; ald, aldehyde; alc, alcohol.