A CONVENIENT MEANS FOR EXAMINING SOME ENZYMOLYTIC AND COMPLEXING REACTIONS[1]

M. Mazurek and A. S. Perlin

Prairie Regional Laboratory
National Research Council
Saskatoon, Saskatchewan
Canada

Received October 30, 1961

Thermometric measurement of the vapor pressure difference between a solution and the pure solvent (i.e., of the heat of vaporization) (1, 2, 3) provides a highly satisfactory means for determining the molecular weight of many organic and inorganic compounds. With the instruments and techniques now available (3-6) such determinations may be made very rapidly on the micro-scale. Since changes in molecular weight are readily detectable also under these favorable circumstances, we have found it convenient to follow thermometrically the course of some reactions that are accompanied by changes in the number of effective particles present.

Results obtained for the action of invertase on sucrose and of β-amylase on glycogen illustrate the usefulness of the method for examining some enzymic hydrolysis reactions*. Measurements were made with a thermoelectric "osmometer" (Mechrolab Inc., Mountain View, California), the general procedure being that used for the determination of molecular weight. Resistance settings ($\triangle R$) could be read directly, and syringes mounted in the measurement chamber of the instrument permitted rapid application of the solution and solvent to the thermistors. In carrying out the experi-

---

[1] Issued as N.R.C. No. 6576.

* A wide variety of such reactions is known (e.g., 7) and the method appears to be generally applicable to those that are homogeneous and do not involve the formation of volatile products.

ments a drop each of the reaction mixture and solvent was suspended on the appropriate thermistor bead at 37° C and, after an initial period for manipulation and equilibration (2-3 minutes), changes in resistance were noted. The concentration of effective particles present at a given time was determined by reference to a plot of ΔR vs. molarity, obtained with a known solute under the same conditions. Blank determinations were made also with solutions of the individual reactants. For rapid hydrolyses (0.5 hours or less) a single drop of the solution sufficed; for longer periods measurements were made at chosen intervals using a new drop each time. (When an unusually high concentration of enzyme was present the latter procedure prevented the formation of a film on the surface of the drop).

The decrease observed in the apparent molecular weight of sucrose (0.03-0.06 M solutions) treated with invertase reached a limiting value equal to that calculated for conversion of the disaccharide to an equivalent each of D-glucose and D-fructose; the quantity of enzyme used was sufficient to promote complete inversion in 0.5 to 1.0 hours. Under these conditions, the rate found within the range 25-85% hydrolysis corresponded strictly to that of a first-order reaction (with respect to sucrose concentration). With lower proportions of enzyme the rates deviated from unimolecularity and, as expected (8, 9), approached zero order. Polarimetric measurements lead to the same conclusions provided, however, that suitable corrections are made for the complex mutarotation effects that accompany the inversion (9, 10). The thermometric data therefore provide an independent check on the kinetics of invertase action, since they are related to the number of particles present in solution and not to chemical constitution.

The hydrolysis of glycogen by β-amylase as measured with the osmometer was compared with data obtained from copper-reducing equivalents of the reaction mixture. Calculated values for percent maltose by the two methods were in close agreement: e.g., in one experiment the values at 40, 60 and 120 minutes, respec-

tively, were 27.9, 30.3 and 33.0 (osmometer); 29.0, 30.5 and 33.0 (reducing power).

In both of these, as well as other enzymolysis reactions, the usual procedures require periodic withdrawal of samples of the reaction mixture, destruction of the enzyme, and analysis by an appropriate method. With the osmometer, however, highly precise measurements may be made continuously without interrupting the reaction, and require only minute amounts of material.

Another type of reaction, examined briefly, is complex formation between borate and polyols. There was little evidence of complexing when boric acid was used. Possibly this was due to a balance of counter-effects; since boric acid behaves as an unionized compound, a decrease in the number of particles by complexing could be offset by the ionic character (11) of the complex itself. With sodium tetraborate (solutions of which were found to give ΔR values almost six times that calculated for the uncharged compound) and several polyols, variable decreases in ΔR occurred. Equilibrium was reached rapidly over a wide concentration range (borate, 0.02-0.1 M; polyol, 0.02-0.6 M), and the observed degree of complexing paralleled that found conductimetrically (11) - e.g., glyceritol $\ll$erythritol $\ll$arabitol $<$mannitol.

ACKNOWLEDGMENT

The authors thank Dr. H. C. Ehrmantraut for helpful information.

REFERENCES

1. Hill, A. V. Proc. Roy. Soc. London, Series A. 127, 9 (1930).
2. Blades, E. J. J. Sci. Instr. 11, 223 (1934).
3. Brady, A. P., Huff, H. and McBain, J. W. J. Phys. Coll. Chem. 55, 304 (1951).
4. Meuller, R. J. and Stolten, J. H. Anal. Chem. 25, 1103 (1953).
5. Neumayer, J. J. Anal. Chim. Acta 20, 519 (1959).
6. Higuchi, W. I., Schwartz, M. A., Rippie, E. G. and Higuchi, T. J. Phys. Chem. 63, 996 (1959).

7. "The Enzymes". Edited by P. D. Boyer, H. Lardy and K. Myrbäch. Academic Press, New York. Vols. 4 and 5 (1960).

8. Brown, A. J. J. Chem. Soc. 81, 373 (1902).

9. Michaelis, L. and Menten, M. L. Biochem. Z. 49, 333 (1913).

10. Hudson, C. S. J. Am. Chem. Soc. 30, 1564 (1908).

11. Böeseken, J. Advances in Carbohydrate Chem. 4, 189 (1949).