Sodium Azide, an Efficient Inhibitor of Protein Diazotization

The coupling of diazonium salts with aromatic compounds has been widely used for the introduction of haptenic groups or other substances e.g., radioactive material into protein[1,2]. The resulting azo compounds are strongly colored and the intensity of the color is a guide to the progress of the reaction. The color generally develops immediately after the solutions containing protein and the diazonium salt are mixed but the reaction may not be completed for 24 h[3]. Therefore, the reaction must be terminated after an appropriate interval in order to achieve a constant extent of diazotization of the protein.

The reaction is commonly terminated by addition of aqueous resorcinol in an amount equal to a hundred-fold molar excess over the aromatic amine[4]. However, resorcinol is poorly soluble and may precipitate proteins from the solution. During a search for an acceptable substitute we found sodium azide to be an efficient inhibitor of protein diazotization. Sodium azide is highly soluble even in high concentrations and in usally employed amounts has no effect on protein solubility.

The inhibitory effect of sodium azide was studied by preparing 0.5 ml aliquots of pooled human serum containing various amounts (1–20 mg/ml serum) of sodium azide (Fisher Scientific, New Jersey) and chilling them in ice. A solution of sulfanilic acid (AR Grade, Mallinckrodt, St. Louis) was prepared by dissolving 100 mg sulfanilic acid in 10 ml 1N hydrochloric acid. Solutions of sulfanilic acid (8 ml), 10% sodium nitrite (8 ml), 0.2 *M* pH 8.6 borate buffer (8 ml) and 1 *N* sodium hydroxide (7.5 ml) were transferred into separate test tubes and chilled in ice. The solutions of sulfanilic acid and sodium nitrite were mixed for 1 min to prepare diazotized sulfanilic acid (DSA). The solutions of sodium hydroxide and borate buffer were then mixed and the mixture was added to the solution of DSA. The pH of the resulting solution was checked and, if necessary, adjusted to 8.6 with additional 1 *N* sodium hydroxide. 2 ml of the freshly prepared DSA (containing the equivalent of approximately 5 mg sulfanilic acid) pH 8.6 were added to each aliquot of serum and mixed by 4–5 inversions of the test tube. An orange color developed immediately but its intensity decreased with increasing concentration of sodium azide. The aliquots were kept for 15 min in ice and for a further 12–16 h at room temperature. An assessment of the extent of the diazotization of proteins was made by spectrophotometric determination of the absorbance at 352 μ. The absorbance of serum diazotized in the presence of various concentrations of sodium azide is illustrated in the Figure. At the employed concentrations neither the solution of DSA nor the solution of serum and sodium azide had perceptible absorption at 352 μ. Complete inhibition of the diazotization of serum proteins occurred at a concentration of sodium azide of 0.75 mg/ml of the reaction mixture. Lesser inhibition of the reaction occurred with lower concentrations of azide as determined by the change in absorbance (Figure).

In a subsequent experiment, increasing amounts of sodium azide were added to 2 ml aliquots of freshly prepared solutions of DSA pH 8.6. Each aliquot was then added to a 0.5 ml volume of serum and the reaction allowed to proceed for 12–16 h. It was again found that sodium azide inhibited the diazotization of protein. The extent of inhibition for similar amounts of sodium azide corresponded to that achieved in the previous experiment. Complete inhibition occurred at a concentration of sodium azide of 0.75 mg/ml of the reaction mixture. By contrast, the absorbance of serum proteins coupled with the DSA did not decrease during incubation in the presence of sodium azide in concentrations up to 25 mg/ml of the reaction mixture. Therefore, sodium azide presumably caused no uncoupling of sulfanilic acid from serum proteins.

Sodium azide probably inhibits the diazotization of proteins by reacting with the diazonium ion[5]. The reaction of the azide with the diazonium ion occurs at approximately equimolar concentrations. Our findings indicate that sodium azide is an efficient inhibitor of the diazotization of protein. Its usefulness is enhanced by its ready availability and low cost.

However, the addition of sodium azide to biological materials can render them unsuitable for diazotization. An indiscriminate use of azide as a preservative e.g., of commercial reagents, is therefore to be avoided[6].

Résumé. L'azide de sodium est un inhibiteur très efficace de la réaction de diazotation. Il réagit avec l'ion de diazonium à des molarités approximativement égales.

M. Nourbakhsh and A. Baumgarten

Department of Laboratory Medicine, Yale University School of Medicine and Department of Clinical Laboratories, Yale-New Haven Hospital, New Haven (Connecticut 06504, USA), 5 June 1972.

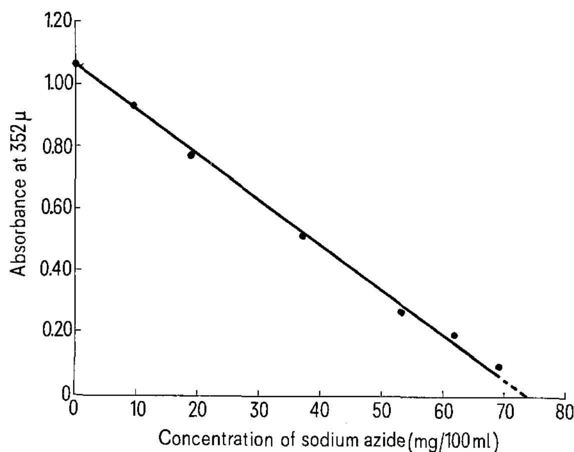

Figure illustrates the decreased absorbance at 352 μ of a solution of serum which has been allowed to react with a constant amount of diazotized sulfanilic acid in the presence of various concentrations of sodium azide.

1 A. Baumgarten, G. J. H. Melrose and W. J. Vagg, Experientia *23*, 884 (1967).

2 A. Baumgarten, G. J. H. Melrose and W. J. Vagg, Analyt. Biochem. *24*, 243 (1968).

3 D. H. Campbell, J. S. Garvey, N. E. Cremb and D. H. Sussdorf, in *Methods in Immunology*, 2nd ed. (W. A. Benjamin, New York 1970), p. 133.

4 C. A. Williams and M. W. Chase, in *Methods in Immunology and Immunochemistry* (Academic Press, New York 1967), vol. 1, p. 123.

5 J. D. Roberts and M. C. Caserio, in *Basic Principles of Organic Chemistry* (W. A. Benjamin, New York 1965), p. 890.

6 This work was partly supported by grant No. 5-SO1-RR-05358-10 U.S. Public Health Service, Washington, D.C.