STABILITY OF THE IODINE-CARBON BOND OF RADIOACTIVE IODOALBUMIN IN THE 50 KV ELECTRON BEAM

ARNOLD M. SELIGMAN, JACOB S. HANKER, ARLENE R. SEAMAN, and HANNAH E. SILBERSTEIN. From the Departments of Surgery and Radiology, Sinai Hospital of Baltimore, and the Johns Hopkins University School of Medicine, Baltimore

In order to develop reagents for histochemistry with the electron microscope, it is important to increase the electron opacity of the reagents by incorporating elements with a high atomic number. Iodine with atomic number of 53 and atomic weight of 126.9 is readily incorporated into aromatic nuclei of various reagents. However, reagents containing three iodine atoms were surprisingly ineffective in increasing the electron opacity in histochemical methods for functional groups of macromolecules (1). Although failure to raise significantly the total density of the macromolecules is the most likely cause, a possible explanation for the results could have been loss of iodine from tissue sections under the influence of the 50 kv electron beam on the carbon-iodine bond in a very high vacuum. In order to test this hypothesis, the loss of radioactivity on exposing radioactive iodoalbumin in a methacrylate section in the electron microscope was determined over a total exposure of 20 minutes. Since radioactive iodinated albumin contains iodine attached to aromatic rings of tyrosine and tryptophan (2), this compound served as a good commercial source of organically bound radioactive iodine for testing the hypothesis.

Radioactive iodoalbumin (462 μc per mg) was precipitated from solution (1 ml) with 10 volumes of acetone. The protein was collected with a filter, air-dried, and 3.3 mg were suspended in butyl methacrylate containing 10 per cent methyl methacrylate in a gelatin capsule (00) and the methacrylate polymerized using 2,2′-azo bis(2-methyl propionitrile) as a catalyst (3, 4). The albumin settled to the bottom of the capsule during polymerization. The block was trimmed and 0.2 μ sections (pale yellow interference color) were cut with a Porter-Blum microtome. The sections were mounted on a carbon-stabilized formvar membrane on an Athene type grid. The radioactivity of the section was measured in a 2 inch well detector with a Baird atomic No. 8100 spectrometer under conditions yielding sample counts better than ten times background. An RCA-EMU$_3$ model 3E electron microscope was used. The specimen was scanned with the electron beam at full intensity for various time intervals and the radioactivity measured after each exposure to the electron beam. The time intervals of scanning were 2, 5, and 10 minutes. No significant loss of radioactivity was noted in this time as shown for one experiment in Table I.

Since this experiment demonstrates that iodinated aromatic phenols and amines can tolerate a 50 kv electron beam without loss of iodine for the time needed for ordinary electron microscopic observation, we cannot escape the conclusion that histochemical reagents containing much more than three atoms of iodine will be required to significantly enhance the opacity of cellular protein constituents with methods that demonstrate functional groups of macromolecules. However, for non-stoichiometric reactions, such as in the demonstration of enzymatic activity, three atoms of iodine may be sufficient for good contrast.

TABLE I

Total exposure time	Loss of initial activity
min.	*per cent*
2	3.5
7	2.9
17	6.2

This investigation was supported by a research grant (CY-2478) from the National Cancer Institute, National Institutes of Health, Department of Health, Education and Welfare, Bethesda.

Received for publication, July 5, 1962.

BIBLIOGRAPHY

1. SEAMAN, A. R., HANKER, J. S., and SELIGMAN, A. M., *J. Histochem. and Cytochem.*, 1961, **9**, 596.
2. FINE, J., and SELIGMAN, A. M., *J. Clin. Inv.*, 1944, **23**, 720.
3. KARMARKAR, S. S., BARRNETT, R. J., NACHLAS, M. M., and SELIGMAN, A. M., *J. Am. Chem. Soc.*, 1959, **81**, 3771.
4. SHIPKEY, F. H., and DALTON, A. J., *J. Applied Physics*, 1959, **30**, 2039.