

Classics

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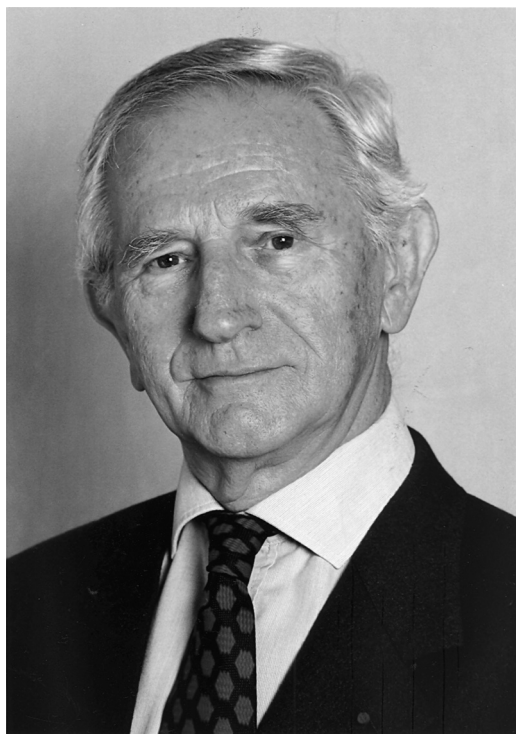
Isolation and Characterization of Antibodies: the Work of Michael Sela

Isolation and Characterization of Active Fragments Obtained by Cleavage of Immunoglobulin G with Cyanogen Bromide

(Cahnmann, H. J., Arnon, R., and Sela, M. (1966) *J. Biol. Chem.* 241, 3247–3255)

Recovery of Specific Activity upon Reoxidation of Completely Reduced Polyallanyl Rabbit Antibody

(Freedman, M. H., and Sela, M. (1966) *J. Biol. Chem.* 241, 5225–5232)



Michael Sela

Michael Sela was born in Tomaszów Mazowiecki, Poland, in 1924. He grew up in the courtyard of a textile factory and intended to study fibers but ended up researching macromolecules when, as a graduate student, he joined the laboratory of Ephraim Katchalski at the Weizmann Institute of Science. Katchalski was exploring the use of polyamino acids as protein models, and Sela became involved in the synthesis of poly-L-tyrosine, poly-3,5-diiodotyrosine, and poly-p-amino-L-phenylalanine. Later on, he aided in the synthesis of polytryptophan and polycyclohexylalanine. For his dissertation, Sela looked at the azo derivatives of some aromatic poly- α -amino acids (1).

After receiving his Ph.D., Sela joined the laboratory of *Journal of Biological Chemistry* (JBC) Classic author Christian Anfinsen (2) at the National Institutes of Health in Bethesda. During this postdoctoral fellowship, Sela looked at the selective cleavage of protein chains by trypsin at arginine residues after lysine residues were reversibly blocked by carbobenzoxy groups (3). He also explored reductive cleavage of ribonuclease, followed by blocking of the sulfhydryl groups with

iodoacetic acid (4). While doing these experiments, Sela and Anfinsen decided to leave part of the reduced ribonuclease unblocked to see if it could reoxidize properly and regain its enzymatic activity. The enzyme showed a total recovery of activity (5), demonstrating that the three-dimensional structure of a protein is the result of its amino acid sequence. Anfinsen went on to receive the 1972 Nobel Prize in chemistry for his research on the connection between the amino acid sequence and the biologically active conformation of ribonuclease.

Sela returned to the Weizmann Institute in 1957. He became interested in immunogenicity and antigenic specificity after reading Karl Landsteiner's book, "The Specificity of Serological Reactions," and learning that gelatin probably is not antigenic because it contains no tyrosine. Intrigued, Sela explored the possibility of increasing the antigenicity of gelatin by attaching tyrosine peptides and showed that limited tyrosylation enhanced immunogenicity without

significantly changing specificity, whereas more extensive tyrosylation converted gelatin into a potent immunogen, provoking antibodies mainly to tyrosyl peptides (6).

As a result of these studies on tyrosylated gelatin, Sela assumed that gelatin was not necessary for immunogenicity and started synthesizing numerous linear and multichain polyamino acids and testing them for immunogenicity. With these synthetic antigens, he was able to elucidate the molecular basis of antigenicity and learned that it was possible to prepare synthetic immunogens leading to antibodies of essentially any specificity. He also was able to distinguish between conformational (conformation-dependent) and sequential determinants (7) and showed that the same peptide may lead to antibodies recognizing its sequence or recognizing an epitope defined by conformation.

These experiments led to Sela's interest in isolating antibodies, and his laboratory succeeded in preparing an analog of the Fab dimer by splitting the IgG molecule with cyanogen bromide rather than with proteolytic enzymes. As reported in the first JBC Classic reprinted here, Sela and his colleagues treated rabbit immunoglobulin G with cyanogen bromide in 0.3 M HCl, resulting in the cleavage of about half of its methionyl peptide bonds. They extracted a 5 S fragment from the reaction mixture by gel filtration and found that the fragment retained its full capacity to precipitate antigen. From these experiments, Sela concluded that CNBr preferentially cleaves the methionyl bonds in the Fc portion of IgG and that the major fragment obtained still has the combining properties of a divalent antibody (8).

Sela eventually determined that poly-DL-alanylation of immunoglobulin resulted in total reduction and solubilization without affecting their biological properties (9). In the second JBC Classic reprinted here, Sela extends this investigation to polyalanyl rabbit antibodies, creating rabbit antibodies to bovine serum albumin enriched with more than 800 DL-alanine residues per molecule. He found that the molecules were completely soluble in neutral aqueous buffers after total reduction and exposure to 8 M guanidine hydrochloride. After reoxidation, the antibody preparations exhibited a recovery of at least 68% of the antigenic determinants present in the untreated antibody molecules. These results suggested that differences in specificity exhibited in antibody molecules are due to differences in the primary amino acid sequence of the constitutive polypeptide chains. They also confirmed the hypothesis that no genetic information other than that present in the amino acid sequence of the polypeptide chains is required for the correct conformation of a protein molecule as complex as immunoglobulin G.

Sela continues to study immunology using synthetic antigens at the Weizmann Institute. His work led to the discovery of the genetic control of the immune response and the design of vaccines based on synthetic molecules. He also has become interested in the possibility of fighting the autoimmune disease experimental allergic encephalomyelitis (EAE) with synthetic analogs of the molecules in the myelin sheath of the brain, which are capable of provoking the disease.

Sela served as chairman of the Weizmann immunology department (1963–1975), vice president of the Institute (1970–1971), dean of the biology faculty (1970–1973), president of the Institute (1975–1985), and finally, deputy chairman of the board of governors (1985–2004). Currently, he is the W. Garfield Weston Professor of Immunology at the Weizmann Institute.

Sela has received many awards and honors for his contributions to science. These include the Israel Prize in the Natural Sciences (1959), the Rothschild Prize in Chemistry (1968), Germany's Emil von Behring Prize (1973), the Gairdner Foundation International Award (1980), France's Institut de la Vie Prize (1984), Germany's Commander's Cross of the Order of Merit Award (1986), France's Officier de l'Ordre de la Légion d'honneur (1987), UNESCO's Albert Einstein Golden Medal (1995), the Interbrew-Baillet Latour Health Prize of Belgium (1997), and the Wolf Prize in Medicine (1998). He is a member of 15 academies of science in various countries, including the U.S. National Academy of Sciences.¹

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¹ For more information on Michael Sela's research, see his JBC Reflections (10).

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