Advanced Techniques in Biological Electron Microscopy II

Specific Ultrastructural Probes

Edited by

J. K. Koehler

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With Contributions by

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With 105 Figures

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Preface

The use of the term "advanced" in the title of this book is somewhat arbitrary and very much relative with respect to time. Many techniques which were considered at the "cutting edge" of ultrastructural methodology just a few years ago are now routinely used in numerous laboratories. One could cite freeze-fracture, cryothin sectioning, or indeed most of the field of scanning electron microscopy as concrete examples. Thus the use of the term "advanced techniques" must be interpreted with regard to the present state of the art, and is useful only in informing the potential reader that this volume is not a primer to be used as an initial introduction into basic biological electron microscopy. Many excellent volumes have filled that niche in the past few years, and it is not intended that this modest book be a complete compendium of the field. Furthermore, any limited selection of papers on advanced techniques necessarily reflects the preferences and arbitrary whims of the editor, thereby excluding many equally important procedures which the knowledgeable reader will readily identify.

The first volume of this series appeared approximately five years ago and illustrated techniques which were thought to represent advanced and yet basically morphological methods for gaining increased ultrastructural information from biological specimens. The present volume, on the other hand, stresses techniques which provide specific physicochemical data on the specimens in addition to the structural information. The future importance of fine structural investigations would seem to be strongly dependent on our ability to adopt such methods to help answer some of the outstanding questions in cell biology.

Three of the contributions of this volume deal with the use of various surface probes having specific affinities for cell surface molecules. The utilization of labeled lectins to explore cell surfaces has grown explosively during the past few years, and is discussed in the chapter by G. L. NICOLSON. Antibody labels have also become a very powerful specific probe of surface activities and are treated in the chapter by W. D. PERKINS and J. K. KOEHLER. S. S. BROWN and J. P. REVEL deal with the use of these and other types of labeled probes in the scanning electron microscope. The use of these methods has already contributed some very important new information to improve our concepts of antigen processing, cell fusion, and exocytosis, to mention just a few examples. The area of scanning electron microscopy is further represented by the chapter on low temperature preparations contributed by P. ECH-

LIN. The localization of highly labile substances in biological materials continues to be a nagging problem and considerable promise for a solution seems to lie in such cryo techniques. Another procedure that will be useful for such investigations involves the use of hydrated specimens which is discussed from the point of view of electron diffraction as well as electron microscopic studies by S. W. HUI and D. PARSONS. Finally, in the area of nucleoprotein fine structure research, two of the most elegant procedures are presented in chapters by J. LAKE, J. FERGUSON and R. W. DAVIS. The first of these is an account of the detailed structure of the ribosome as determined from nascent antibody labeling studies, and the second describes the use of heteroduplex analysis in genetic mapping research.

My sincere appreciation is extended to the authors of these chapters for their time-consuming efforts and patience, to the staff of the Springer-Verlag for their dedication to the production of the highest quality scientific publications, and to Ms. DORIS RINGER for help in the editorial processing of the manuscripts.

Seattle, February 1978 JAMES K. KOEHLER

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S. S. BROWN and J.-P. REVEL (With 3 Figures)

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Ultrastructural Localization of Lectin Receptors

G. L. NICOLSON

A. Introduction

Lectins, proteins, or glycoproteins that bind to specific sequence(s) in oligosaccharides, have been extensiveley used in cell biology to examine certain aspects of cell surface and membrane structure and function (reviews: LIS and SHARON, 1973; NICOLSON, 1974 c; 1975; SHARON and LIS, 1975). The classical use for lectins is cell agglutination, and agglutinability has been found to vary with antigenic type, state of infection, growth (cell density and contact), stage of the cell cycle, during embryonic development, after oncogenic transformation, etc. (MÄKELÄ, 1957; LIS and SHARON, 1973; NICOLSON, 1974 c). In addition to their assets as cell oligosaccharide structural probes, lectins have found extensive use in membrane biology in studying the number, distribution, turnover, display and mobility, and biogenesis of membrane glyco-components. Quantitative lectin binding (using ^{125}I-, ^{3}H-, or ^{63}Ni-labels) has been successfully used to measure the number of lectin receptors, their affinities, and kinetics of lectin association (and dissociation), and fluorescent lectins have been utilized for low-resolution lectin receptor localization at the level of light microscopy. The variety of ultrastructural techniques for lectin receptor localization utilizing electron microscopy will be discussed further.

Many lectins possess important biological properties. Lectins can affect cell transport systems (ISSELBACHER, 1972; CZECH and LYNN, 1973), mimic hormone action (CUATRECASAS and TELL, 1973; CZECH and LYNN, 1973), stimulate mitosis (MÖLLER, 1970; POWELL and LEON, 1970; JANOSSY and GREAVES, 1971; ANDERSSON et al., 1972) and platelet release (MAJERUS and BRODIE, 1972), secretion of migration factors (SCHWARTZ et al., 1970), and histamine (SIRAGANIAN and SIRAGANIAN, 1974) as well as other processes (NICOLSON, 1974 c). They have been found to inhibit cell growth (DENT, 1971; INBAR et al., 1972; RALPH and NAKOINZ, 1973), movement (FRIBERG et al., 1972), phagocytosis (BERLIN, 1972), delayed hypersensitivity (LEON and SCHWARTZ, 1969), allograph rejection (MARKOWITZ et al., 1969) and fertilization (OIKAWA et al., 1973; 1974).

The number of different kinds of lectins available to the investigator is tremendous. For example, MÄKELÄ (1957) listed over 260 in his review and

TOMS and WESTERN (1971) compiled data for over 500 different lectins. However, most of these lectins have not been purified and characterized, so their use in electron-microscopic localization experiments is minimal. The most suitable lectins for electron microscopy and some of their properties are listed in Table 1. It is apparent from the diversity of different lectin-binding specificities that a variety of highly specific, but different cell surface sites can be probed by the use of a few lectins.

Several techniques for lectin-binding site localization are presented here along with their respective advantages and disadvantages, and simplified methods of synthesis of the ultrastructural probes will be detailed. The advice and help of many experts has been used to prepare this chapter, and their assistance is gratefully acknowledged (see Acknowledgements).

B. Purification of Lectins

Most lectins are obtained from plant or other sources as complex mixtures of proteins, lipids, and other contaminants. Before they can be used for electron-microscopic localization studies, it is advisable (or absolutely necessary in some cases) to purify them further by conventional or affinity techniques. Although the literature is rife with conventional lectin purification schemes using salt precipitation, gel chromatography, ion exchange chromatography, preparative electrophoresis, and others, the emphasis here is on affinity purification of lectins. Affinity purification procedures have been developed for most of the lectins in Table 1, and some of the more widely used affinity chromatography techniques are listed in Table 2. We have found for our own studies that affinity purified lectins yield lower backgrounds and greater binding specificity than all but those most highly purified by conventional procedures.

Affinity purified lectins can be radiolabeled before the ultrastructural probes are constructed. Although not done routinely, this serves three purposes: (1) the kinetics and extent of labeling can be determined independently on a large number of cells; (2) any loss of lectin label during the electron-microscopic preparation steps can be monitored; and (3) the ratio of lectin to ultrastructural marker (ferritin, peroxidase, hemocyanin, etc.) can be determined. A list of suitable isotope-labeling techniques for lectins has been presented in Table 2 of SHARON and LIS (1975), the most popular radiolabels being ^{125}I and ^{3}H.

C. Purification of Markers

Various ultrastructural markers have been used to identify lectins bound to their target sites. Some of these markers are covalently linked to lectins

Table 1. Some lectins suitable for electron microscopy

Origin	Common name or abbreviation	Mitogenic activity	Approximate molecular weight	Subunits	Blood group specific	Inhibitory saccharides	Key references
Canavalia ensformis (jack bean)	Con A (Concanavalin A)	+	55,000	2	—	α-*D*-Man, α-*D*-Glc	SUMNER and HOWELL (1936); SO and GOLDSTEIN (1967 a, b); AGRAWAL and GOLDSTEIN (1967, 1968); PORETZ and GOLDSTEIN (1968, 1970)
Dolichos biflorus (horse gram)	DBA (Dolichos biflorus agglutinin)		135,000	4	A	α-*D*-GalNAc	BIRD (1951); ETZLER and KABAT (1970); FONT et al. (1971)
Glycine max. (soybean)	SBA (Soybean agglutinin)	– [a]	110,000	2	—	*D*-GalNAc, *D*-Gal	WADA et al. (1958); LIS et al. (1966, 1969, 1970); GORDON et al. (1972 a, b); LIS and SHARON (1972); CATSIMPOOLAS and MEYER (1969)
Helix pomatia (snail)	—	?	100,000	~6	A	α-*D*-GalNAc	HAMMARSTRÖM and KABAT (1969, 1971); UHLENBRUCK et al. (1970 b); ISHIYAMA and UHLENBRUCK (1972)

[a] SBA is reported to be mitogenic to neuraminidase-treated lymphocytes (NOVOGRODSKY and KATCHALSKI, 1971)

Table 1 (continued)

Origin	Common name or abbreviation	Mitogenic activity	Approximate molecular weight	Subunits	Blood group specific	Inhibitory saccharides	Key references
Lens culinaris (lentil)	LCA-A	+	I= 60,000	2	—	α-*D*-Man, α-*D*-Glc	Howard and Sage (1969); Tichá et al. (1970); Toyoshima et al. (1970); Howard et al. (1971); Paulová et al. (1971 b); Young et al. (1971)
	LCA-B (Lens culinaris agglutinin)	?	II= 42,000	2	—	α-*D*-Man, α-*D*-Glc	
Limulus polyphemus (horseshoe crab)	—	?	400,000	18	—	Sialic acid	Marchalonis and Edelman (1968); Nowak and Barondes (1975)
Lotus tetragonolobus	Lotus A	+	I=120,000	4	H(O)	α-*L*-Fuc	Yariv et al. (1967); Kalb (1968); Marchalonis and Edelman (1968)
	Lotus B	?	II= 58,000	2	H(O)	α-*L*-Fuc	
	Lotus C	?	III=120,000	4	H(O)	α-*L*-Fuc	
Phaseolus vulgaris (red kidney bean)	L-PHA	+	I=140,000	4?	—	*D*-GalNAc,	Rigas and Osgood (1955); Coulet et al. (1956); Allan et al. (1969); Rigas and Head (1969); Dahlgren et al. (1970); Allen and Crumpton (1971, 1973); Schumacher et al. (1971); Miller et al. (1973)
	H-PHA (Phytohemagglutinin)	+	II=140,000	4?	—	*D*-GalNAc	
Pisum sativum (pea)	Pea agglutinin	+	I= 54,000	2	—	*D*-Man, *D*-Glc	Entlicher et al. (1969, 1970); Paulová et al. (1971 a)
		+	II= 53,000	2	—	*D*-Man, *D*-Glc	

Table 1 (continued)

Origin	Common name or abbreviation	Mitogenic activity	Approximate molecular weight	Subunits	Blood group specific	Inhibitory saccharides	Key references
Ricinus communis (castor bean)	RCA_I	–	I = 120,000	4	—	β-*D*-Gal,	KABAT et al. (1947); DRYSDALE et al. (1968); WALDSCHMIDT-LEITZ and KELLER (1969, 1970); GÜRTLER and HORSTMANN (1973); NICOLSON and BLAUSTEIN (1972); NICOLSON et al. (1974); OLSNES et al. (1974)
	Ricin, RCA_{II} (Ricinus communis agglutinin)	–	II = 60,000	2	—	*D*-Gal, *D*-GalNAc	
Sophora japonica	—	?	133,000	—	A+B	β-*D*-GalNAc	MILLER and BOYD (1967); PORETZ and TIMBERLAKE (1972); PORETZ et al. (1974)
Triticum vulgaris (wheat germ)	WGA (Wheat germ agglutinin)	–	23,000	2		$(D\text{-GlcNAc})_2$, sialic acid	BURGER and GOLDBERG (1967); UHLENBRUCK et al. (1968, 1970 a); BURGER (1969); BIDDLE et al. (1970); LEVINE et al. (1972); ALLEN et al. (1973); GREENAWAY and LEVINE (1973)
Ulex europaeus	UEA_I	+	I – 170,000	—	H(O)	*L*-Fuc,	MATSUMOTO and OSAWA (1969, 1970)
	UEA_{II} (Ulex europaeus agglutinin)	–	II = 170,000	—	H(O)	$(D\text{-GlcNAc})_2$	

Table 2. Affinity purification of some lectins

Lectin	Specificity	Affinity adsorbent	Elutant	References
Con A	α-*D*-glc α-*D*-Man	Sephadex G-50	*D*-glc or sucrose	AGRAWAL and GOLDSTEIN (1967)
			glycine-HCl, pH 2	OLSON and LIENER (1967)
DBA	*D*-galNAc	polyleucyl (A+H substance)	*D*-galNAc	ETZLER and KABAT (1970)
		Con A-Sepharose	α-Me-*D*-Man	GOLDSTEIN et al. (1973)
LCA	α-*D*-glc α-*D*-man	Sephadex-G100	*D*-glc	TICHÁ et al. (1970); HOWARD et al. (1971); YOUNG et al. (1971)
P. vulgaris	*D*-galNAc	Thyroglobulin-Sepharose	Glycine-HCl, pH 3	MATSUMOTO and OSAWA (1972)
PHA		Con A-Sepharose	α-Me-*D*-Man	GOLDSTEIN et al. (1973)
PSA	α-*D*-glc α-*D*-man	Sephadex-G150	Glycine-HCl, pH 2	ENTLICHER et al. (1970); TROWBRIDGE (1974)
RCA_I	β-*D*-gal	Sepharose 4B	*D*-gal	TOMITA et al. (1972)
		Biogel A 1.5 m	*D*-gal or lactose	NICOLSON and BLAUSTEIN (1972); NICOLSON et al. (1974)
SBA	*D*-gal *D*-galNAc	N-ε-aminocaproyl-β-*D*-galactopyranodylamine-Sepharose	*D*-gal	GORDON et al. (1972 a+b)
		Con A-Sepharose	α-Me-*D*-man	GOLDSTEIN et al. (1973)
SJA	α-*D*-gal	Polyleucyl (A+H substance)	*D*-gal	PORETZ et al. (1974)
UEA	*L*-fuc	*L*-fucose-starch	glycine-HCl, pH 4	MATSUMOTO and OSAWA (1972)
		Tri-N-acetylchitotriose-starch	Glycine-HCl, pH 3	MATSUMOTO and OSAWA (1972)
WGA	$(D\text{-glcNAc})_2$	Ovomucoid-Sepharose	Acetic acid	MARCHESI (1972); NICOLSON et al. (1975 a)
		N-ε-aminocaproyl-β-D-glucopyranosylamine-Sepharose		LOTAN et al. (1973)
		Chitin-Sepharose		BLOCH and BURGER (1974)

while others are bound to target-attached lectins by virtue of the fact they possess the appropriate carbohydrate sequence(s) for lectin binding. Simplified schemes for some markers are given below.

I. Hemocyanin

Busycon canaliculata are usually obtained from the Marine Biological Laboratory (Woods Hole, Mass., U.S.A.), but we have used Pacific keyhole limpets as well. The shell is broken with a hammer in the region of the heart, or a sharp knife is thrust through the foot into the heart, and the animals are placed into large supported funnels. Hemolymph is allowed to drip into a beaker at room temperature, and the debris is removed by low-speed centrifugation (15,000 *g* for 10 min). The supernatant is centrifuged at 59,000 *g* (Beckman-Spinco rotor Model Ti50 at 30,000 rpm or equivalent) for 15 to 30 min at 4° C to concentrate the hemocyanin (to 50 – 70 μg/ml) without pelleting. The resulting hemocyanin solution is about 90% pure at this point, and it is further purified by gel chromatography on Sepharose-2B or Biogel-A 0.5 m. The material is usually stored sterile at 4° C. It should not be frozen.

II. Ferritin

Many commercial grades of ferritin are available. Only a few are ready for ultrastructural work such as electron-microscopic grade ferritin (Polysciences, Inc., Warrington, Penna.; Immuno-diagnostic Systems, Solana Beach, Calif.; Sigma Chemical, St. Louis, Mo.). All other grades must be further purified before they can be used as electron-microscopic markers (HSU, 1967). Be aware that several manufacturers sell lyophilized ferritin that is partially denatured. If ferritin is frozen or lyophilized, *it cannot be used for electron microscopic work.* Orders for ferritin should contain directions to ship air mail in temperature-controlled polyfoam containers to prevent accidental freezing during transit.

Ferritin (2X or 6X crystallied, but *not* EM grade) can be purified by the following scheme: crystallization in 5% cadmium sulfate, precipitation in 50% ammonium sulfate, and ultracentrifugation.

1. Cadmium Sulfate Crystallization

Ferritin is diluted to 10 mg/ml in 2% ammonium sulfate and 0.35 volumes of 20% $CdSO_4$ are slowly added with stirring. The solution is stored at 4° C until crystals form and settle (usually 0.5 – 2 days); longer storage results in loss by adherence to glass. The ferritin crystals are carefully pelleted