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Laser light scattering determination of size and dispersity of synaptosomes and synaptic vesicles isolated from squid (*Loligo pealei*) optic lobes

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Abstract. Quasi-elastic laser light scattering has been used to investigate the size and dispersity of synaptosomes and synaptic vesicles isolated from optic lobes of the squid *Loligo pealei*. Synaptosomal fractions were highly polydisperse ($\mu_2/\bar{\Gamma}^2 = 0.5$) and the mean diameter ($\bar{d}$) ranged from 0.5–2.0 μm. Size distribution histograms yielded two major components – smaller particles ($\bar{d} \sim 300-700$ nm) and a larger group of particles ($\bar{d} \sim 1{,}500-5{,}000$ nm). The heterogeneity of the synaptosomal particles detected in solution is in agreement with published data obtained using electron microscopy. Purified synaptic vesicle fractions also yielded complex particle size distribution data. A component with a mean diameter in the range 150–250 nm was detected, though a smaller particle ($\bar{d} \sim 40-110$ nm) dominated the scattering signal. This smaller particle closely resembles in size the electron lucent vesicles seen in the majority of squid optic lobe nerve terminals when examined by electron microscopy. Osmotically-induced shrinkage and swelling of the synaptosomes was detected. Depolarization by veratridine (1.0×10^{-4} *M*) did not result in a detectable change in the size of synaptosomal particles.

Key words: Quasi-elastic laser light scattering, synaptic vesicles, synaptosomes, squid (*Loligo pealei*)

Introduction

Isolated nerve endings (synaptosomes) prepared from invertebrate CNS tissues are characterized by the almost complete absence of postsynaptic attachments (Breer and Jeserich 1984). Also, for cephalopod and insect synaptosomes, which have been most thoroughly investigated, the high density of CNS cholinergic neurones is reflected in the high proportion of cholinergic terminals in synaptosomal preparations (Dowdall and Whittaker 1973; Dowdall and Simon 1973; Dowdall 1974; Breer and Jeserich 1984; Breer 1981a, b; Dwivedy and Sattelle 1984). These invertebrate preparations appear to yield relatively pure fractions of cholinergic nerve endings.

Nevertheless, detailed electron microscopical investigations of cephalopod synaptosomes reveal multiple subtypes, falling into distinct categories based on size and ultrastructural characteristics (Haghighat et al. 1984). The prospect of rapidly and non-invasively determining the physical heterogeneity of both isolated nerve terminals and synaptic vesicles prepared from them, prompted the present laser light scattering characterization of subcellular fractions prepared from optic lobes of the squid *Loligo pealei*.

Quasi-elastic laser-light scattering (QELS) provides a rapid non-destructive probe of the hydrodynamic size of macromolecules and membrane-bound vesicles (Cummins and Pike 1977; Sattelle et al. 1982). This technique has been used to study sarcoplasmic reticulum vesicles (Arrio et al. 1974; Selser et al. 1976), isolated neurosecretory vesicles from chromaffin cells (Green et al. 1978) and lipsosomes (Day et al. 1977). To date QELS has not been applied in a detailed investigation of synaptic vesicles and nerve endings purified from brain tissue. Conventional, total intensity light scattering measurements have been employed to monitor volume changes in nerve ending particles (Kamino et al. 1973).

By simultaneously measuring hydrodynamic size and the scattered light intensity, many of the difficulties encountered in the interpretation of absorbance measurements are avoided. Early applications of QELS to in vivo nerve terminals of arthropods detected a slow increase in light intensity fluctuations (Shaw and Newby 1972; Piddington and Sattelle 1975; Sattelle et al. 1975; Englert and Edwards 1978) as a result of exposure to high-potassium saline. Here in addition to

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providing estimates of particle size distributions for synaptosomes and isolated synaptic vesicles, we also investigate the effects of veratridine-induced depolarization on nerve terminals isolated from the squid optic lobes.

Methods

Synaptosome preparation

Synaptosomes were prepared from squid optic lobes using the procedure of Pollard and Pappas (1979). Fourteen squid optic lobes were homogenized at 0 °C as a 20% (w:v) solution of 1.0 *M* sucrose containing 10.0 m*M* HEPES (pH 7.4) by 8–10 complete strokes of a glass-on-glass Wheaton (40 ml) homogenizer (B pestle). The homogenate was centrifuged at 10,000 r.p.m. (13,000×*g*) for 1 h at 4 °C in a Sorvall RC2B centrifuge using an SM-24 rotor. The synaptosomal pellicle was removed by aspiration and, unless otherwise indicated, was resuspended in 5.0 ml of either 1.0 *M* buffered sucrose, or artificial seawater to provide suspensions of crude synaptosomes. Media for resuspending the synaptosomes were previously filtered through a Millipore (0.2 µm diameter pore) filter.

The resuspended synaptosomes were disaggregated by 8 complete strokes of a glass-on-glass Blessing homogenizer. Resuspended crude synaptosomes and dispersed synaptosomes were further diluted 1:50 (v:v) into the final suspension medium (volume 2.0 ml) in a 10 mm-sided square plastic cuvette (Curtis-Matheson Scientific).

Synaptic vesicles

A crude preparation of synaptic vesicles was prepared by diluting the synaptosomal fraction 1:10 in filtered (0.2 µm Millipore) 10.0 m*M* HEPES buffer (pH 7.4). The preparation was rehomogenized in the Wheaton homogenizer (pestle B) and then agitated with a magnetic stirrer for 45 min at 0 °C. This lysed preparation was centrifuged at 19,000 r.p.m. (30,000×*g*) for 20 min at 4 °C in a Sorvall RC2B centrifuge using an SM24 rotor, and the supernatant provided a crude synaptic vesicle preparation. This supernatant was pelleted by centrifugation at 35,000 r.p.m. (55,000×*g*) for 2 h at 4 °C using the 50.Ti fixed-angle rotor of a Beckman L5-65 ultracentrifuge. The resulting pellet (P_3) was resuspended in 100 µl axoplasm buffer (modified from Morris and Lasek (1980) of the following composition (in m*M*): aspartate, 400; taurine, 130; betaine, 70; $MgCl_2$, 13; EGTA, 10; $CaCl_2$, 3; HEPES, 40 (pH 7.2 adjusted with 1.0 *M* KOH).

The resuspended vesicles were layered on to a sucrose step-gradient (0.4 *M*; 0.6 *M*; 1.0 *M* buffered sucrose) and centrifuged for 2 h at 4 °C using the SW 50.1 swing-out rotor of a Beckman L5-65 ultracentrifuge. Earlier studies showed the 0.4 *M* sucrose fraction to be enriched in acetylcholine-containing vesicles (Dowdall and Whittaker 1973). Purified vesicles concentrated in the 0.4 *M* sucrose band were removed, and from this stock they were diluted into axoplasm buffer which had previously been filtered (Millipore 0.2 µ*M* pore diameter).

Quasi-elastic laser light scattering

Using a Coulter model N4MD multiangle particle analyser equipped with a 5 mW HeNe laser, quasi-elastic laser light scattering (QELS) was used to measure diffusion coefficient, particle-size distribution and total scattered intensity from samples of synaptosomes and synaptic vesicles. This instrument computes the autocorrelation function of the intensity fluctuations ($g^2(\tau)$), which, for a heterogeneous population of scatterers could be written as an expansion about $\bar{\Gamma}$ (the mean of the distribution of the decay rates weighted by the intensity scattered from each species) such that

$$\tfrac{1}{2}\ln[g^2(\tau)-1] = -\bar{\Gamma}\tau + \frac{\mu_2\tau^2}{2!} - \frac{\mu_3\tau^3}{3!}, \qquad (1)$$

where μ_2 and μ_3 (the second and third cumulants) measure the width and skewness of the weighted decay rate distribution (Koppel 1972; Berne and Pecora 1976). The quantity $\mu_2/\bar{\Gamma}^2$ is the normalized variance of the diffusion coefficient distribution and provides a measure of the sample polydispersity (Koppel 1972). From $\bar{\Gamma} = \bar{D}K^2$ (where K is the scattering vector, given by $\frac{4\pi n}{\lambda_0}\cdot\sin\frac{\theta}{2}\Big)$, an average diffusion coefficient ($\bar{D}$) can be determined, and using the Stokes-Einstein equation ($D = K_B T/3\pi\eta d$) an average diameter ($\bar{d}$) of an equivalent spherical scatterer can be estimated.

The Coulter N4MD size distribution processor (SDP) computer programme utilizes CONTIN, a programme developed at the European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany, for the constrained regularization of linear equations (Provencher et al. 1978; Provencher 1979, 1982 a, b). The calculated particle size distribution is approximated by a histogram with the height of each column adjusted by parameters in the fitting programme to achieve the best fit to the observed autocorrelation function. Both SDP and cumulants analysis have been used in the present study. The time required to collect data for a single autocorrelation function was between 10 s and 200 s. Control samples of latex spheres (d = 40 nm, 100 nm and 500 nm) were measured.

Table 1. Diffusion coefficient, size and dispersity of synaptosomes and synaptic vesicles isolated from optic lobes of the squid (*Loligo pealei*)

Fraction	Prep	$\bar{D}_{20,w}$ [10^{7} cm^2 s^{-1}]	$\bar{d}$ [nm]	$\mu_2/\bar{\Gamma}^2$	d_1 [nm]	%	d_2 [nm]	%	d_3 [nm]	%
Synaptosomes (resuspended)	1	0.026 ± 0.001 (3)	1,863 ± 45 (3)	0.46 ± 0.03 (3)	3,760 ± 1,384	28	663 ± 347	66	160 ± 7	6
	2	0.045 ± 0.002 (4)	933 ± 44 (4)	0.55 ± 0.02 (4)	3,748 ± 417	69	320 ± 35	31	–	–
	3	0.053 ± 0.006 (3)	1,114 ± 61 (3)	0.43 ± 0.04 (3)	4,369 ± 625	47	402 ± 66	53	–	–
Synaptosomes (resuspended dispersed)	1	0.079 ± 0.002 (5)	521 ± 53 (5)	0.48 ± 0.08 (5)	1,451 ± 685	66	287 ± 65	34	–	–
Synaptic vesicles (crude preparation)	1	0.32 ± 0.01 (4)	133 ± 3.2 (4)	0.21 ± 0.02 (4)	100 ± 40	55	231 ± 73	45	–	–
	2	0.33 ± 0.003 (6)	132 ± 1.2 (6)	0.21 ± 0.02 (6)	45 ± 6	17	171 ± 28	83	–	–
Synaptic vesicles (purified)	1	0.46 ± 0.15 (3)	94 ± 30 (3)	0.22 ± 0.09 (3)	104 ± 45	100	–	–	–	–
	2	0.46 ± 0.12 (3)	93 ± 28 (3)	0.17 ± 0.07 (3)	99 ± 15	100	–	–	–	–
	3	0.47 ± 0.04 (8)	92 ± 11 (8)	0.11 ± 0.02 (8)	105 ± 21	100	–	–	–	–

Values are expressed as mean ± one standard deviation. The number of measurements are shown in brackets. Concentrations were as follows in mg ml^{-1} protein: synaptosomes, 1.12; crude vesicles, 0.42; purified vesicles, 0.37

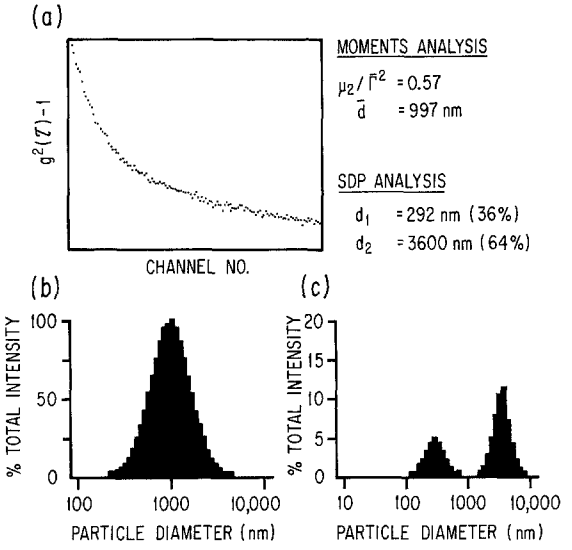

Fig. 1. a Intensity autocorrelation function derived from squid optic lobe synaptosomes in artificial seawater plotted as $g^2(\tau)-1$ against channel number (each channel represents 10 μs). The polydispersity index ($\mu_2/\bar{\Gamma}^2$) is determined from the decay rate ($\bar{\Gamma}$) of the correlation function. The average hydrodynamic diameter ($\bar{d}$) of the synaptosomes is estimated from the Stokes-Equation using $\bar{D}_{20,w}$. **b** Particle size distribution of crude synaptosomes estimated by fitting distribution to the Pearson V distribution function (cf. McCally and Bargeron 1977). **c** Particle size distribution of synaptosomes determined utilising the CONTIN programme (cf. Provencher 1982a, b)

Chemicals

Veratrine sulphate was obtained from Sigma Chemical Co. (St. Louis, Mo.) and purified veratridine was the gift of Prof. G. Holan (CSIRO, Melbourne, Australia). All other chemicals used were of analytical grade.

Results

Size and dispersity of synaptosomes

Aliquots of the dispersed synaptosomal fraction were diluted 1:50 into filtered (0.2 μm Millipore) seawater or buffered sucrose and examined in the N4 Coulter particle analyzer at either 15° or 20 °C ($\theta = 90°$). In all cases a monotonically decaying intensity autocorrelation function was observed (Fig. 1). Diffusion coefficient ($\bar{D}_{20,w}$), particle diameter ($\bar{d}$) and polydispersity ($\mu_2/\bar{\Gamma}^2$) values obtained from correlation data are summarized in Table 1. Values for $\bar{D}_{20,w}$ in the range $0.026-0.079\times10^{-7}$ cm^{2}s^{-1} were observed. The synaptosome fraction was polydisperse ($\mu_2/\bar{\Gamma}^2$ values ranged from 0.43 – 0.55) and $\bar{d}$ was of the order of 0.5 – 2.0 μm. When samples were subjected to SDP analysis two major particles appeared to dominate the scattering signal – a smaller particle ($\bar{d} \sim 287-663$ nm), and a larger particle ($\bar{d} \sim 1{,}451-4{,}369$ nm). In some preparations a third particle of the order of one hundred microns was detected, but this contributed only a small fraction to the total scattered intensity. The mean diameter of synaptosomes subjected to gentle rehomogenization was at the lower end of the range presumably due to the removal of aggregates (Table 1).

Effects of varying osmolarity

To test if the synaptosomes were sealed, aliquots of the stock fraction were diluted (1:50) in buffered sucrose in the range 1.0 *M* to 0.4 *M*. Significant increases in $\bar{d}$ were noted in both 0.6 *M* and 0.4 *M* buffered sucrose, when compared to 1.0 *M* buffered sucrose (Fig. 2). No significant difference in total scattered intensity was

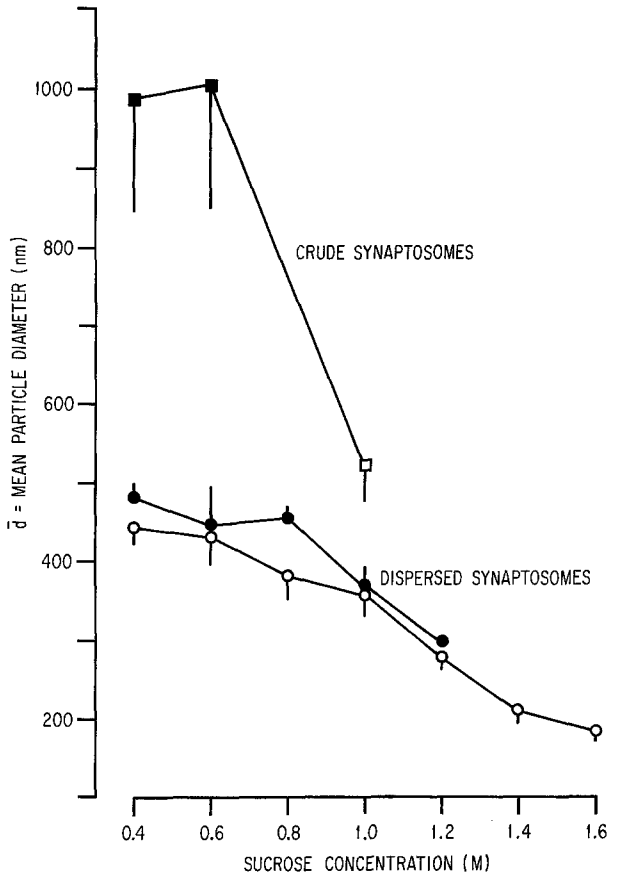

Fig. 2. Effects of osmolarity on the average diameter ($\bar{d}$) and total scattered intensity derived from squid synaptosomes in HEPES (10 mM, pH 7.4) buffered sucrose solutions

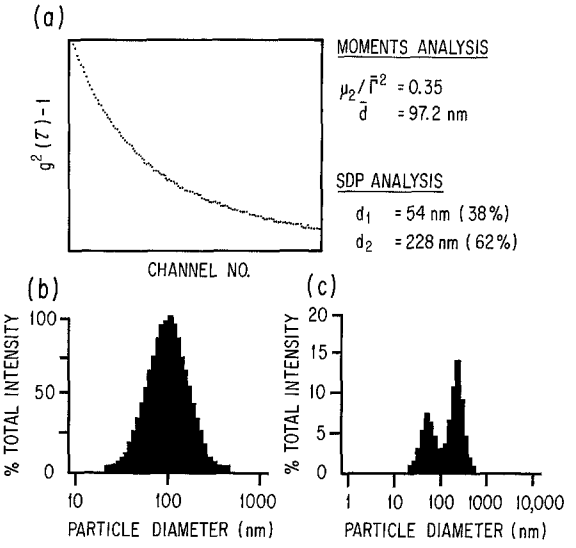

Fig. 3. a Intensity autocorrelation function derived from crude synaptic vesicles prepared from squid optic lobes. Vesicles are suspended in filtered axoplasm buffer and data are plotted as $g^2(\tau)-1$ against channel number (each channel represents 50 μs). The average hydrodynamic diameter ($\bar{d}$) and polydispersity index ($\mu_2/\bar{\Gamma}^2$) of the vesicles are determined from the decay rate ($\bar{\Gamma}$) of the correlation function. The average hydrodynamic diameter ($\bar{d}$) of the vesicles is estimated from the Stokes-Einstein equation, using $\bar{D}_{20,w}$. **b** Particle size distribution of synaptic vesicles estimated by fitting distribution to the Pearson V distribution function (cf. McCally and Bargeron 1977). **c** Particle size distribution of synaptic vesicles determined using the CONTIN programme (cf. Provencher 1982 a, b)

detected between the three samples. Similar findings were obtained in two similar series of experiments.

Effects of veratridine

Veratridine depolarizes nerve terminal membranes releasing neurotransmitter (Minchin 1980). No maintained changes in size, dispersity and total scattered intensity were detected on the addition of either veratrine ($n=3$) or purified veratridine ($n=2$) (to a final concentration of $1.0\times10^{-4}M$ in each case) to synaptosomes resuspended in filtered natural or artificial seawater ($n=3$). Initial readings were taken within 2–5 min of the addition of the alkaloid. In one preparation maintained in sucrose (1.0 M), veratrine ($1.0\times10^{-4}M$ final concentration) did result in a transient increase in total scattered intensity.

Size and dispersity of synaptic vesicles

Crude synaptic vesicles were prepared by lysis of synaptosomes. The polydispersity ($\mu_2/\bar{\Gamma}^2=0.21$) was lower than that seen for synaptosomes (Table 1). Also, the average diffusion coefficient ($\bar{D}_{20,w}$ approximately $0.32\times10^{-7}\,cm^2\,s^{-1}$) was about an order of magnitude higher than that obtained for synaptosome samples. Analysis of light scattering data by the SDP programme indicated the presence of two discrete particles – a larger particle ($\bar{d}\sim171-231$ nm diameter) and a smaller particle ($\bar{d}\sim45-100$ nm).

Density gradient centrifugation was used to further purify the vesicles and an example of intensity autocorrelation data is shown in Fig. 3. These fractions were less polydisperse ($\mu_2/\bar{\Gamma}^2=0.11-0.22$) than crude synaptic vesicles, and when resuspended in axoplasm buffer yielded diameters of around 90 nm diameter (Table 1). Similar values (97 ± 10 nm diameter) were obtained for vesicles suspended in 0.4 M sucrose, though vesicles suspended in 1.0 M sucrose were significantly smaller (80 ± 2 nm diameter). Using SDP analysis of the data obtained from purified synaptic vesicles a single population of particles was normally detected (Table 1).

Discussion

The physical heterogeneity of isolated squid (*Loligo pealei*) optic lobe nerve terminals shows some similarities with findings reported from electron microscopy, but the distinctive bimodal distribution of scattering particle diameters detected by quasi-elastic laser light scattering (QELS) has not hitherto been reported for invertebrate synaptosomes. Ultrastructural studies on locust synaptosomes reveal many profiles of around 1.0 μm with the bulk of the terminals falling within the range reported for *Octopus* supraoesophageal lobes, where a mean diameter of 0.93 μm is reported (Jones 1967). Using data from *Octopus* optic lobe synaptosomes, the same author obtained a slightly smaller average diameter (0.62 μm). Our finding of synaptosomal size heterogeneity is in agreement with the observations of Haghighat et al. (1984), who describe five subtypes of nerve endings in the intact optic lobe of *Loligo pealei*, corresponding to five distinct types of terminal seen in the synaptosome fraction. Two of these categories, which probably correspond to two kinds of photoreceptor nerve endings in the cortex of the optic lobe, are made up of terminals often much larger than a micron in diameter (Haghighat et al. 1984). The other three distinct types of terminal seen by electron microscopy are smaller in size, and correspond to the nerve endings seen in the medulla region. It is therefore possible that the bimodal distribution of particle sizes seen in light scattering experiments could represent the two size categories of nerve terminals noted in ultrastructural studies. In this context it is of interest to note that Redburn and Thomas (1979) isolated two morphologically-distinct synaptosomal fractions from rabbit retina. One fraction was enriched in relatively large diameter photoreceptor cell synaptosomes, whereas the other contained smaller synaptosomes derived from conventional sized nerve endings from the inner plexiform layer.

Examination of vesicle fractions by QELS also revealed heterogeneity of scatterers, though particle diameters in the range 45 – 231 nm are in broad agreement with ultrastructural findings. In *Octopus* synaptosomes agranular vesicles exhibited a wide range of diameters (15 – 120 nm) in agreement with values for intact *Octopus* nerve tissue. However, the great majority of these vesicles were in the size range 25 – 50 nm diameter (Jones 1967). More recently ultrastructural studies on optic lobe terminals reveal the presence of clear vesicles ($\bar{d} \sim 48 - 72$ nm) though some dense core vesicles ($\bar{d} \sim 100$ nm), are also detected (Haghighat et al. 1984). The detection of scatterers as large as 200 nm in diameter in light scattering experiments on crude vesicle preparations may be due to the presence of vesicle aggregates, or contamination by larger subcellular particles. Differences between vesicle sizes determined by light scattering and electron microscopy may also be attributable to an increase in vesicle diameter under the conditions used for isolation, and/or shrinkage resulting from the preparative procedures employed in electron microscopy.

A rapid change in total scattered intensity, probably representing an increase in large angle scattering has been shown to accompany the calcium-sensitive secretory release process in neurohypophysial nerve terminals of vertebrates (Salzberg et al. 1983, 1985). Using QELS in conjunction with isolated squid synaptosomes we have monitored changes in the average scattered intensity over the period for which the intensity autocorrelation data was collected (10 – 200 s). The absence of any striking change in either scattered intensity or hydrodynamic size during veratridine-induced depolarization of synaptosomes does not preclude the existence of a transient intensity change of the type recorded from intact vertebrate neurosecretory nerve endings. Further experiments are needed on a shorter timescale to test whether or not such light scattering changes can be reproduced in isolated nerve endings (synaptosomes).

Laser light scattering provides a rapid non-destructive probe of hydrodynamic size and polydispersity of both isolated synaptosomes and synaptic vesicles. Particle size distributions of purified vesicles and nerve endings should facilitate the development of improved protocols for purification of subclasses of nerve ending particles and vesicles by further subcellular fractionation.

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