ANGULAR LIGHT-SCATTERING STUDIES ON ISOLATED MITOCHONDRIA

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ABSTRACT

Angular light-scattering studies have been carried out on suspensions of isolated rat liver mitochondria. The angular scatter pattern has a large forward component, typical of large particles. Changes in dissymmetry and in the intensity of light scattered at 90° have been correlated with changes in optical density during the course of mitochondrial swelling and contraction. Such changes can be measured at mitochondrial concentrations much below those required for optical density measurements. Changes in mitochondrial geometry caused by factors "leaking" from mitochondria, not detectable by optical density measurements, have been demonstrated by measuring changes in dissymmetry. Angular light-scattering measurements therefore offer the advantages of increased sensitivity and of added indices of changes in mitochondrial conformation.

Size, shape, and volume changes of isolated mitochondria have been studied in a number of laboratories in order to ascertain the properties of the membranes and the relationship of size changes to respiratory metabolism. Such studies have utilized both direct visual observations including phase contrast microscopy (1–5) as well as photometric observations of the light-scattering properties of mitochondrial suspensions (3, 5–11). With few exceptions (12, 13) the photometric observations have been confined entirely to studies of the optical density of mitochondrial suspensions under different conditions. Changes in optical density have been correlated with changes in mitochondrial volume and mitochondrial water content (3, 5, 6, 8, 9, 14–17). By this procedure mitochondria have been shown to exhibit behavior approximating that predicted by osmotic theory (5, 9). In addition, optical density measurements have shown that under isotonic conditions mitochondria undergo swelling, which may be caused by certain specific reagents, such as thyroxine, glutathione, inorganic phosphate, Ca^{++}, and oleic acid (*e.g.*, 11, 15). Optical density measurements have also been used to follow decreases in mitochondrial volume in the presence of ATP (15, 17, 18).

In this paper angular light-scattering studies on isolated mitochondria are reported. While available light-scattering theory is not sufficiently developed to permit interpretation in detail of the data obtained from particles having the size and geometry of mitochondria, we have found that angular light-scattering measurements afford at least two advantages over simple measurements of optical density in the study of isolated mitochondria: (*a*) The high sensitivity of the measurements of light scattered at an angle of 90° to the incident beam allows studies to be carried out at much lower concentrations of mitochondria than would be feasible by conventional turbidity measurements. (*b*) Measurements of light scattering as a function of angle reflect changes in mitochondrial size and shape which are not measurable by optical density studies.

METHODS

The mitochondria utilized for these studies were isolated in 0.25 M sucrose by methods described previously (18). The experiments reported were carried out with rat liver mitochondria, though similar experiments performed with rat heart sarcosomes gave qualitatively the same results.

Angular scatter measurements were made with an Aminco model 4–6000 light-scattering microphotometer. A cylindrical microcell with a diameter of 13 mm. was used. In order to reduce scattering errors due to this small radius of curvature, the cell was centered within a 40-mm.-diameter cylindrical cell containing freshly distilled water. The water surrounding the microcell was thermostated at 25 ± 0.5°C. The beam of incident light was defined by a pair of collimating slits which resulted in a beam width of 2.8 mm. at the exit slit and an angular divergence of 1°. The beam of scattered light viewed by the photometer was 3 mm. wide by 2.5 mm. high as defined by a second collimating slit system. All measurements reported were performed with unpolarized incident light of a wave length of 436 mμ, but qualitatively similar results were also obtained with wave lengths of 546 and 579 mμ. The intensity of scattered light as a function of angle was measured in a horizontal plane, with the emergent beam considered the reference angle of 0°. No attempt was made to obtain an absolute calibration of the intensity of the scattered light and this is therefore reported in artbitrary units.

Optical density measurements were made in 15 × 100 mm. test tube cuvettes in the Beckman model B spectrophotometer, at 520 mμ. In experiments in which angular light-scattering measurements were compared with optical density measurements, same test suspension of mitochondria was u each assay. During the intervals between pho determinations, the mitochondrial test suspensions were kept in a water bath thermostated at 25 ± 0.5°C. Any system showing evidence of agglutination or the inclusion of extraneous debris was discarded. Examination under the phase contrast microscope of samples of randomly selected systems showed them to contain only particles of mitochondrial dimensions.

RESULTS

Scattering Characteristics of Mitochondria

The intensity of the light scattered by freshly isolated rat liver mitochondria immediately after suspension in various media was measured as a function of angle. Results are expressed in terms of the intensity of the light scattered at 90° from the incident light (I_{90}), the intensity of scattered light as a function of angle, and also in terms of the ratio of the intensity of light scattered at two supplementary angles (dissymmetry).

(*a*) I_{90}: A linear relationship between I_{90} and the mitochondrial concentration, expressed as mg. biuret nitrogen per ml. was observed up to 0.08 mg. nitrogen per ml. Above this concentration the values for I_{90} are only slightly lower than would

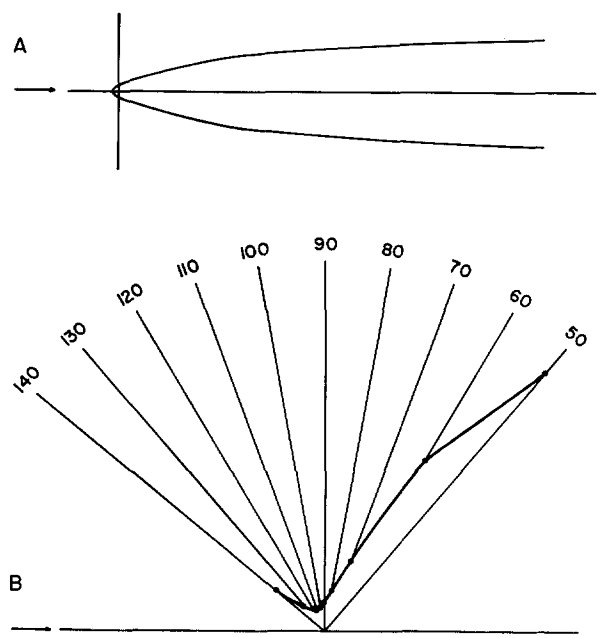

FIGURE 1

Scatter envelope of mitochondria. Mitochondria were isolated and suspended in 0.88 M sucrose. Wave length 546 mμ. Temperature 22°. *A* shows the full scatter envelope. *B*, with the scale 100 times greater than *A*, emphasizes the area from 50° to 140°. Arrow points in the direction of the incident light.

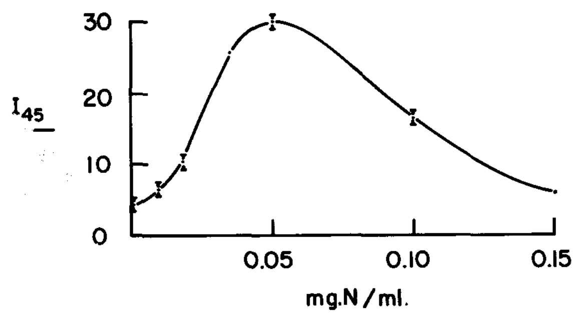

FIGURE 2

The effect of mitochondrial concentration on the dissymmetry at 45°. The medium was 0.125 M KCl, containing 0.02 M tris, pH 7.4. Wave length 436 mμ. Temperature 22°. Mitochondrial concentration (abscissa) is expressed in terms of mg. biuret nitrogen/ml. Where available, the span of values from several experiments is shown.

be predicted. Measurements of I_{90} provide not only much greater sensitivity but also a much larger range of mitochondrial concentration over which effects can be studied. With the light-scattering photometer used in these studies, concentrations varying from 0.001 mg. nitrogen per ml. to well over 0.15 mg. nitrogen per ml. could be readily studied. On the other hand the useful range of optical density measurements is very limited and lies between approximately 0.015 and 0.1 mg. nitrogen per ml.

(*b*) *Scatter Envelope:* As can be seen in Fig. 1*A*, the angular scattering by suspensions of mitochondria is almost entirely in the forward direction, as would be expected by particles of such large size. This large forward scatter is primarily due to the rescattering of light by secondary scattering events (19). Fig. 1*B* shows the details of the angular scattering in the interval from 50° to 140°. It is evident that there is a minimum in the scatter envelope at about 108°. The details of the scatter pattern shown in Fig. 1*B* are qualitatively similar to that shown by solutions of tobacco mosaic virus (20) and suspensions of polystyrene spheres (21).

(*c*) *Dissymmetry:* The dissymmetry at 45° is defined as the ratio of the intensity of light scattered at 45° to the intensity of light scattered at 135° (I_{45}/I_{135}). Fig. 2 shows a distinct maximum in a plot of the dissymmetry at 45° versus concentration. Such a maximum, at about the same mitochondrial concentration, was obtained whether the mitochondria were suspended in 0.125 M KCl, containing 0.02 M tris(hydroxymethyl)aminomethane-HCl (tris) buffer, pH 7.4, or in sucrose, 0.25 or 0.88 M.

Light Scattering as a Function of the Sucrose Concentration of the Medium

It has been shown in earlier studies that mitochondria undergo predictable changes in volume when placed in sucrose solutions of varying osmolarity (9). A comparison of optical density and light scattering by mitochondria in sucrose media of differing concentrations was carried out. A mitochondrial concentration was chosen which allowed accurate optical density readings over the entire range of sucrose concentrations, from 0 to 0.88 M. Optical density readings were made within 30 seconds of the initial mixing of the mitochondrial suspension. This was followed by light-scattering measurements and then a second optical density reading. Insignificant differences were found between the two optical density readings, even at the lowest sucrose concentrations, indicating the stability of mitochondrial volume during the time required for measurements.

As seen in Fig. 3, the changes in the I_{90} closely parallel those obtained with optical density measurements. The general shape of these curves is in good agreement with the results of Tedeschi and Harris (9).

As discussed by these authors, if the wave length and mitochondrial concentration are kept constant, as in these experiments, the optical density varies inversely with the mitochondrial volume and the refractive index of the medium in a complex manner. The drop in the optical density curve at high sucrose concentration would therefore be a reflection of the change in refractive index of the medium when mitochondrial volume had become constant at its minimum value.

The change in dissymmetry at 45° with sucrose concentration is seen to be qualitatively similar to the variations in optical density and I_{90}. Thus, changes in volume produced by changes in the osmolarity of the medium produce parallel and comparable changes in all three types of measurements.

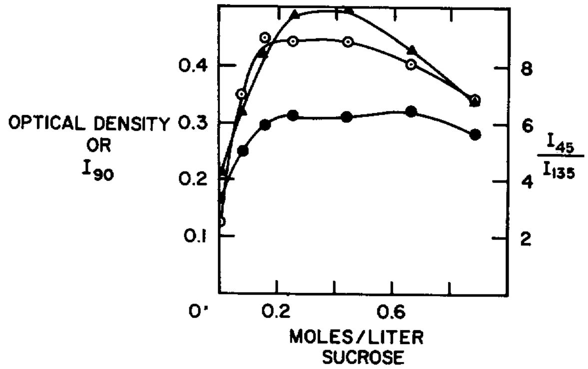

FIGURE 3

Osmotic swelling of mitochondria. Mitochondrial concentration was approximately 0.03 mg. biuret nitrogen/ml. Wave length 436 mμ. Temperature 22°. Values for I_{90} are given in arbitrary units. △---△ represents I_{90}; ⊙---⊙, optical density; and ●---●, dissymmetry at 45°.

Measurements of Swelling and Contraction by Light Scattering and Optical Density

In Fig. 4 it is seen that changes in the I_{90} caused by spontaneous swelling of mitochondria and subsequent contraction by ATP are very similar to previously described changes in optical density (22). It should be noted, however, that the relative changes in the I_{90} are greater than those in the optical density by a factor of about three. At mitochondrial concentrations of 0.001 mg. nitrogen per ml., similar time-course plots were obtained, with changes in the I_{90} greater than 50 per cent after 40 minutes. Thus, angular light-scattering measurements not only permit study of much lower concentrations of mitochondria than do optical density measurements, but also yield much larger amplitudes of response.

Mitochondrial swelling initiated by various specific agents and its reversal by ATP were also studied by light-scattering techniques. Changes in dissymmetry were, for the most part, found to parallel the changes in the I_{90}.

Swelling as a Function of Mitochondrial Concentration

In interpreting light-scattering data so as to obtain absolute molecular weights and sizes of the particles being studied, it is customary to extrapolate the scattering data to zero particle concentration to eliminate the effect of particle-particle interaction on the intensity of scattered light. It was of interest, therefore, to extrapolate data from mitochondria while they were in both swollen and unswollen states.

The mitochondrial concentration was varied over a range from 0.001 to 0.05 mg. nitrogen per ml. The intensity of scattered light at the various angles was measured before and 30 minutes after the addition of the swelling agent. The I_{90} and the dissymmetry were plotted as a function of mitochondrial concentration, as in Fig. 5. Two curves are thereby obtained for each experiment, one representing the unswollen mitochondria, the other the swollen mitochondria.

As seen in Fig. 5*A*, at low mitochondrial concentrations, the linearity between the I_{90} and mitochondrial concentration is retained when the mitochondria are in the swollen state; however, the slope of the line changes. Such changes in slope were observed after spontaneous swelling and after swelling by various specific agents. The initial and final slopes could not be correlated with any specific variables in the system. The plot in Fig. 5*B* demonstrates that the dissymmetries of mitochondria in the swollen and unswollen

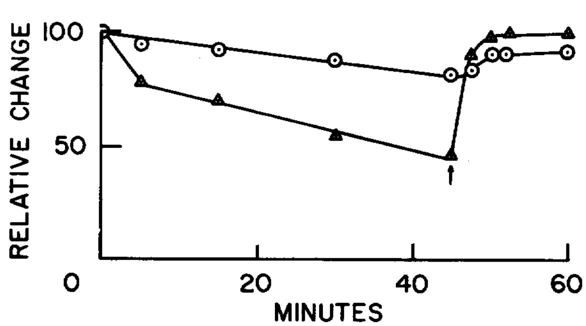

FIGURE 4

Effect of spontaneous swelling and its reversal on optical density and I_{90}. Mitochondrial concentration was 0.02 mg. biuret nitrogen/ml. Wave length 436 mμ. Temperature 22°. At the arrow, additions of 0.005 M ATP, 0.003 M $MgCl_2$, and 2.0 mg. per ml. of bovine serum albumin were made. ⊙——⊙ represents optical density; △——△, I_{90}. Values are normalized as relative changes, with the initial reading given a value of 100 and subsequent readings presented as a percentage of this.

state both extrapolate to the same point at zero mitochondrial concentration.

One interpretation of these data might be that the mitochondria had changed in size, but not in shape. It must be pointed out, however, that the interpretation of data obtained from mitochondria at different concentrations is more complicated than the interpretation of extrapolated data from homogeneous, well defined particles such as proteins. In the latter case, it is assumed that the only variable is the particle concentration and therefore only particle-particle interaction is eliminated by the extrapolation. With mitochondria, however, such an assumption is not valid. When mitochondrial concentration is varied not only is the particle concentration altered, but also the concentrations of proteins, substrates, and cofactors which leak from the mitochondria (23–29). Therefore, any interpretation utilizing data obtained from mitochondria at different concentrations must be viewed with caution.

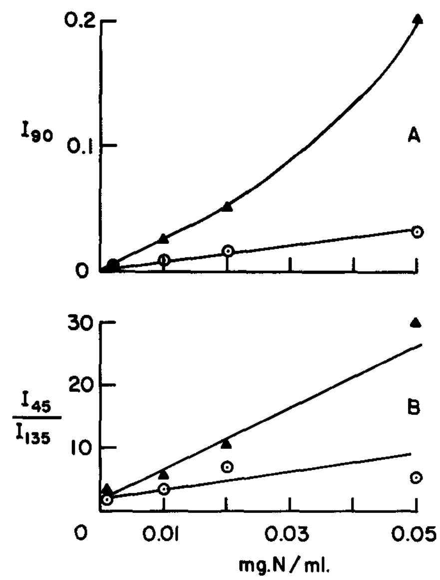

FIGURE 5

Effect of swelling and mitochondrial concentration on I_{90} and dissymmetry at 45°. The medium was 0.125 M KCl, containing 0.02 M tris, pH 7.4. Wave length 436 mμ. Temperature 22°. I_{90} given in arbitrary units △---△ represents initial values, before additions of swelling agent. ⊙---⊙ represents values obtained 30 minutes after the addition of 0.02 M phosphate, pH 7.4. The scale for the abscissa for both plots is the same.

Detection of Factors Leaking from Mitochondria by Dissymmetry Measurements

Several mitochondrial components have been shown to leak from mitochondria into the storage or incubation medium, *e.g.*, nucleotides, substrates, and protein (23–29). Of greatest significance is the C-factor protein, which is apparently required to effect ATP-induced mitochondrial contraction. It was found that a factor(s) leaking from mitochondria at 0° stimulates a change in the dissymmetry of mitochondrial suspensions.

Mitochondria prepared in 0.25 M sucrose were incubated at ice bath temperature for 1 hour. After that time they were sedimented by centrifugation and approximately 80 per cent of the supernatant liquid ("used medium") was removed. The mitochondria were then resuspended in the remaining medium. Aliquots of this suspension were then added to the appropriate media. The final mitochondrial concentration ranged from about 0.02 to 0.05 mg. nitrogen per ml. As seen in Fig. 6*A*, addition of mitochondria to a fresh sucrose medium (control) resulted in swelling, indicated by a drop in the I_{90}. However, there was very little change in the dissymmetry in this period. On the other hand, when the mitochondria were resuspended in the "used medium" containing whatever materials originally leaked from the mitochondria during prior storage, both the I_{90} and the dissymmetry values declined in a parallel manner over a 60-minute period. Thus the dissymmetry measurements indicate the occurrence of changes in mitochondrial shape or size which are not revealed by I_{90} or optical density measurements. They further indicate that the mitochondria lose factors into the medium on storage in sucrose at 0° which affect or control changes in size or shape.

DISCUSSION

At the present time light-scattering theory has not been sufficiently developed to deal with the case of a large, structured particle such as a mitochondrion. However, Mie (30) has obtained a mathematical solution for the intensity of light scattered by a homogeneous sphere of given size and refractive index. For particles with a diameter greater than one-third the wave length of the incident light and a ratio of particle refractive index greater than unity, calculations based on the Mie equations are lengthy and complex (20). While the detailed calculations are available for a number of specific cases (20, 31–37) none are

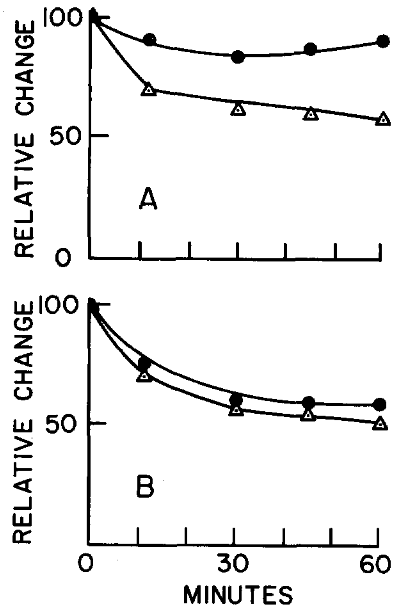

FIGURE 6

Effect of factors liberated from mitochondria on chondria on changes in I_{90} and dissymmetry. Conditions are as explained in text. Wave length 436 mμ. Temperature 22°. Plot *A* demonstrates changes after resuspension of mitochondria in fresh sucrose medium. Plot *B* demonstrates changes after resuspension of mitochondria in "used medium." ●---● represents dissymmetry at 45°; △---△, I_{90}. Values are normalized as relative changes, with the initial reading given a value of 100 and subsequent readings presented as a percentage of this. Abscissa of both *A* and *B* have the same time scale.

available for the size and refractive index range of mitochondria. However, certain features are common to the scattering patterns of all large particles; the typical pattern shows a large forward scatter, and intensity maxima and minima at specific angles (20). The proportion of forward scatter increases and the intensity maxima and minima become more complex as the size and refractive index of the particle increase. The intensity maxima and minima, however, disappear with only a small degree of heterogeneity in particle size (38). The scattering envelope of the mitochondrial suspension shown in Fig. 1 displays the large forward scatter expected for a particle of its size, although only a single minimum at 108° is evident. Complicated scatter patterns have been observed in suspensions of bacteria, and other cells (38, 39), which have refractive indices comparable to that of mitochondria. The absence of additional maxima or minima in the mitochondrial scatter envelope, therefore, is due probably to a heterogeneity in size rather than to a low refractive index.

At finite concentrations of scattering particles, the light intensity at a given angle is a function of both the particle geometry and the interaction between particles. The effect of particle-particle interaction on the scattered-light intensity may be eliminated by reducing the concentration of scattering particles to sufficiently low values. Under such conditions where the effect of particle-particle interaction is eliminated, the I_{90} decreases linearly as the concentration is decreased. It is this behavior which has been observed for mitochondrial suspensions in the concentration range below 0.08 mg. nitrogen per ml.

It is interesting to compare the concentration dependence of the dissymmetry for mitochondria with the concentration dependence reported for other systems. Examination of Fig. 2 shows that the dissymmetry in the mitochondrial system goes through a maximum and then decreases as the concentration approaches zero. In contrast to this behavior the dissymmetry of tobacco mosaic virus has been reported to increase linearly as the virus concentration approaches zero (20). A similar behavior has been reported for various polyelectrolytes at low ionic strengths (40–42). In addition, in the polyelectrolyte systems the dissymmetry shows both a maximum and a minimum with decreasing polyelectrolyte concentration.

Experiments reported in this paper have demonstrated that the swelling and contraction of mitochondria are accompanied by changes in the I_{90} and dissymmetry, as well as the optical density, of mitochondrial suspensions. Despite the present inability to provide quantitative interpretation, it is clear that changes in the light-scattering parameters do reflect changes in the state of the mitochondria. In osmotic swelling and phosphate-induced swelling the changes in I_{90} and the dissymmetry have been seen to parallel one another.

In general, the relative changes in the I_{90} measurements are considerably larger than the corresponding changes in optical density. The I_{90} thus seems to be the more sensitive index of mitochondrial change. Also, dissymmetry measurements afford an index of change in mitochondrial conformation which is not measurable by optical density or I_{90} determinations. Such measurements reveal the leakage of mitochondrial factors which appear to influence mitochondrial configuration.

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