MITOCHONDRIAL INNER MEMBRANE CHANGES DETERMINED BY CROSS PARTITION.

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Intact beef heart mitochondria and submitochondrial particles have different isopartition points. For intact mitochondria a cross-point is obtained at pH 5.6 and for submitochondrial particles at pH 6.6. ATP but not necessarily ITP lowers the isopartition point in submitochondrial particles, indicating conformational and/or charge changes in the particulate membrane.

INTRODUCTION

In previous investigations we have established that in particles obtained by sonic irradiation of beef heart mitochondria other nucleoside triphosphates than ATP could act as the energy source for succinate-linked NAD^+ reduction (1), but exclusively ATP changed the particle membrane according to light-scattering measurements (2). This apparent discrepancy made us start an investigation of membrane parameters such as surface charge and conformation under different conditions, as measured by a liquid-liquid distribution method. This is the first report from this investigation. When cell organelles are introduced in a two-phase system obtained by mixing aqueous solutions of dextran and PEG they will partition between the two liquid phases and the interphase (3). With a suitable composition of the phase system the distribution will be charge-dependent. The pH-dependent distribution of amphoteres in the presence of two different salts will give a defined cross-point, the isopartition point (4). Isopartition points have been determined for proteins (5,6) as well as for rat liver mitochondria (7) and have values close to the isoelectric points. It has been assumed that changes in the charge and conformation of membranes can be described by changes in the cross-point (8).

MATERIALS AND METHODS

All chemicals used were of reagent grade. Dextran 500, batch 3936, molecular weight M_w = 500 000, was purchased from Pharmacia Fine Chemicals, Uppsala, Sweden. Polyethyleneglycol (PEG), grade Carbowax 4000, molecular weight M_n = 3000 - 3700, was supplied by Union Carbide Chemicals, New York, U.S.A. Ampholine (pH 6 - 8) was bought from LKB-Produkter AB, Bromma, Sweden. Nucleotides

Abbreviation: PEG = Polyethyeneglycol

were bought from Sigma Chemical Co., St. Louis, Mo., U.S.A.
Beef heart mitochondria and submitochondrial particles were prepared as previously described (9,10). Protein content was determined according to Gornall et al. (11). The cross-point experiments were carried out according to Ericson (7) in a phase-system containing 6 % (w/w) Dextran, 6 % (w/w) PEG 4000, 320 mmoles sucrose/kg, 5 mmoles phosphate buffer/kg and 50 mmoles alkalisulphate or 100 mmoles alkalichloride respectively/kg. The biological material was added in a concentration of 2 - 3 mg protein/ml. The separation was carried out in ice-bath. The pH and protein content in the phases were determined according to Ericson (7). Isoelectric focusing experiments were performed in the LKB 8108 Ampholine Electrofocusing Equipment (LKB-Produkter AB, Bromma, Sweden) according to their instruction manual.

RESULTS

The distribution of mitochondria in the two-phase system can be expressed either as the percentage, recovered in the top phase of the total quantity in the system or as the partition coefficient K ($K = C_T/C_B$ where C_T and C_B are the concentrations in the top and bottom phase respectively). Although there is generally an adsorbtion of mitochondria at the interphase, essentially the same cross-point will be obtained regardless of the criterion used (7). From Fig. 1 it is clearly seen that the cross-point for intact

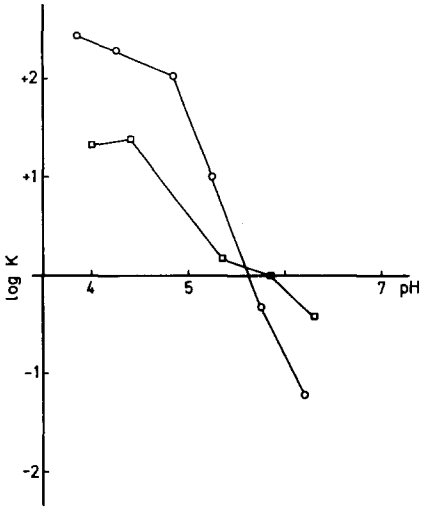

Fig. 1. Distribution of beef heart mitochondria in a Dextran-PEG two-phase system.
The phase-system contained 6 % (w/w) Dextran 500, 6 % (w/w) PEG 4000, 5 mmoles citrate-phosphate-buffer/kg, 320 mmoles sucrose/kg and 50 mmoles Na_2SO_4/kg (□) or 100 mmoles NaCl/kg (o). Mitochondria was added in a concentration of 2 mg protein/ml. The distribution is expressed as the partition coefficient K.

beef heart mitochondria has a pH-value of about 5.6. The observed value of the isopartition point is completely in agreement with the findings of Ericson (7) for rat liver mitochondria.

The results from a similar and typical experiment with submitochondrial particles are depicted in Fig. 2. A cross-point at pH about 6.6 was achieved in this particular case and the same value was obtained when sodiumphosphate buffer or Tris-Cl buffer solutions were employed instead of phosphate-citrate buffer, indicating that the type of buffer had no importance on the isopartition point, but a cross-point at a somewhat lower pH-value was obtained in the presence of alkali salts other than lithium, probably due to differences in the interaction between the cation and the membrane.

The electrofocusing experiment, in a sucrose density and pH gradient,obtained by Ampholine carrier ampholytes, demonstrates, as seen in Fig. 3, that the isoelectric point of the submitochondrial particles has approximately the same pH-value as the isopartition point obtained in the two-phase system (Fig. 2). Data from the electrofocusing experiment seem also to indicate a minor peak corresponding to an isoelectric point at a pH-value of about 7. Experiments with Ampholine carriers giving a pH-value below 6 did not give any detectable peaks.

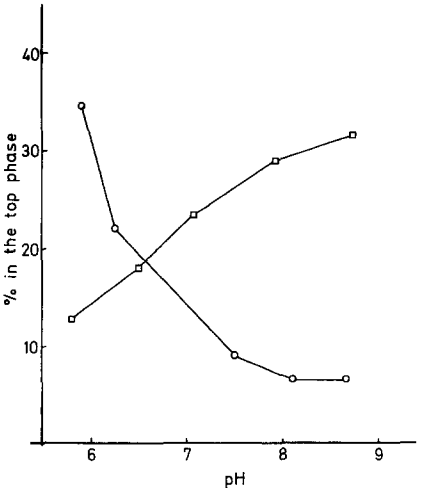

Fig. 2. Distribution of submitochondrial particles in a Dextran-PEG two-phase system.
Experimental conditions as in Fig. 1, but Li_2SO_4 (□) and LiCl (o) was used instead of the sodium salts. The distribution is expressed as percentage of the total quantity found in the top phase.

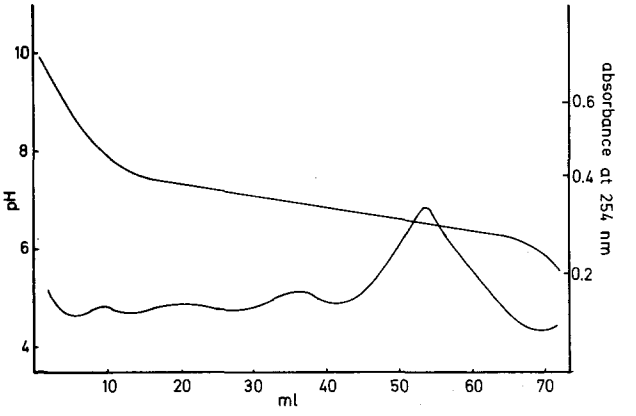

Fig. 3. Isoelectric focusing of submitochondrial particles in an Ampholine gradient, pH 6 - 8. Submitochondrial particles, 1 mg of protein, was added to the light medium during preparation of the sucrose gradient. The electrophoresis was run for 20 h.

When ATP was added to the submitochondrial suspension immediately prior to the two-phase distribution procedure, a remarkable change of the cross-point was observed, as seen in Fig. 4. This cross-point for the particles at a lower pH-value in the presence of ATP could not be fully imitated by an equimolar concentration of ITP. The small differences between untreated and ITP-treated particles might be due to the increased ionic strength of the phase systems. The concentration of phosphate in the systems was too low to counteract any ATP-induced changes of the membrane (2).

DISCUSSION

From the data presented here, there is no doubt that intact mitochondria and submitochondrial particles have different isoelectric points. This is not surprising since the particles consist of mitochondrial innermembrane spheres with an inverted orientation compared to intact mitochondria (12) and the innermembrane has furthermore a "sideness" with e.g. the ATPase located on the matrix side and cytochrome c facing the outside of the membrane (for review, see Harmon et al. (13)). In this context is it worth to notify that Heidrich (14) has demonstrated different electrophoretic mobility between sonicated and non-sonicated innermembrane-matrix fractions of mitochondria. The inside-out fragments obtained by sonication were less negatively charged at the pH used (slightly above neutral). This is in agreement with our findings, where at pH 7.0 the submitochondrial particles

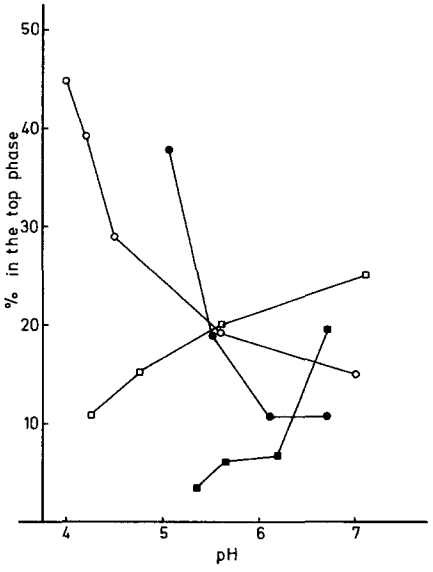

Fig. 4. Distribution of submitochondrial particles in the presence of ATP or ITP.
Experimental conditions as in Fig. 2. 10 mM ATP (open symbols) or ITP (closed symbols) was added to the particles prior to the distribution procedure.

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are closer to the isoelectric point than are intact mitochondria. Both the intact mitochondria and the submitochondrial particles are negatively charged at pH 7.0, as the sulphate containing phases give a higher partition coefficient than those cotaining chloride (3).

It has been suggested that a preparation of submitochondrial particles obtained by sonication consists of 64 % inside-out fragments (15). With sucrose density gradient centrifugation Huang et al. (16) obtained two distinct types of membrane fragments, one of which (the X-fragment) contained what morphologically seemed to be inside-out particles. The other type (the Y-fragment) had a smoother surface but could not be definitely characterized as an outside-out particulate fraction (17). Essentially the same type of fragments has also been described by Astle and Cooper (18). On the other hand Rhodin and Racker (19) have stated that their type of particles (prepared from bovine heart mitochondria in the presence of $CaCl_2$ or $MnCl_2$) were all inside-out. It is not possible from the results presented in this report to evaluate if our Mg^{2+}-ATP-particle preparations are "homogenous" or not.

A second type of particle would be indicated by the small peak in the

electrofocusing experiments (Fig. 3) and it is moreover not known if a cross-point represents a mean value of different populations. On the other hand an innermembrane fraction, with a membrane orientation similar to intact mitochondria, would give a cross-point at about pH 5.3 (7) and thus fall on the other side of the big peak along the pH-axis in Fig. 3. It has been assumed that there are differences between the inner boundary membrane and the crista membrane concerning e.g. the content of cytochromes (20). Moreover particles contain mitochondrial outermembrane fragments (21). However, particles purified by free-flow electrophoresis had an activity of monoamine oxidase (EC 1.4.3.4) which was only 18 % of that of intact mitochondria. The same percental ratio was obtained between our Mg^{2+}-ATP-particle preparation and intact mitochondria as examined by the method of Wurtman and Axelrod (22). This would imply that our preparation contains negligable amounts of outermembranes. From the results of Ericson (7) it is obvious that the outer membrane fraction of rat liver mitochondria has a cross-point at a lower pH than the innermembrane fraction. Further experiments with the two-phase distribution technique will probably give an answer to the question as to whether our preparations consist of two (or more) different membrane populations although at the moment it seems obvious that the inside-out type of innermembrane particles is the most predominant.

From the data presented in Figs. 2 and 4 it is obvious that ATP, but not necessarily ITP, lowers the isopartition point of the submitochondrial particles. This seems to indicate conformational and/or charge changes in the particulate membrane of the same type as presented previously (2), where those changes were supposed to be nucleotide specific.

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