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Conformational changes of a mitochondrial precursor protein on binding to phospholipid vesicles and SDS micelles

A circular dichroism and fluorescence spectroscopy study

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Conformations of an artificial mitochondrial precursor protein pCox IV-DHFR have been analyzed by CD and fluorescence spectroscopy in the presence of (cardiolipin-rich) phospholipid vesicles or SDS micelles. Binding of pCox IV-DHFR to phospholipid vesicles involves a conformational change, which is presequence-dependent, accompanies alteration in the secondary structure of the DHFR moiety, but is different from total unfolding of the polypeptide chain. On the other hand, a conformational change of the fusion protein on binding to the micelles of a positively charged detergent, SDS, is not presequence-dependent.

Mitochondria; Protein import; Precursor unfolding; Protein-lipid interaction; CD; Tryptophan fluorescence

1. INTRODUCTION

Many secretory and organellar proteins have to move through biological membranes to reach their destination. Recent works have suggested that proteins have to be at least partly unfolded during or before translocation across membranes (for reviews see [1,2]). One possible mechanism to achieve a partially unfolded conformation is mediated by acidic phospholipids at target membranes [3–5].

Lipid-induced unfolding has been studied for an artificial mitochondrial precursor protein pCox IV-DHFR, which can be imported by yeast mitochondria in vitro and in vivo [6,7]. This fusion protein uses at least in part the same import pathway as several authentic mitochondrial precursor proteins [8]. Import of the fusion protein into mitochondria has been resolved into two steps: binding to the mitochondrial surface accompanying unfolding of the DHFR moiety to a trypsin-sensitive conformation, and a subsequent ATP-dependent translocation into the matrix [9]. The binding step can be mimicked by incubating the native fusion protein with vesicles containing acidic phospholipids. The fusion protein bound to phospholipid vesicles resembles the translocation intermediate bound to mitochondrial surface: both display increased susceptibility to trypsin digestion [3,4]. Thus the fusion protein bound to phospholipid vesicles offers a model system to biophysically study the transient, unfolded conformation of a precursor protein during its import.

In the present study, conformations of the fusion protein bound to cardiolipin-rich phospholipid vesicles and SDS micelles have been

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Abbreviations: pCox IV-DHFR, a fusion protein consisting of a presequence of cytochrome oxidase subunit IV and mouse dihydrofolate reductase; DHFR, mouse dihydrofolate reductase; CD, circular dichroism; p25, a chemically synthesized peptide corresponding to the entire presequence of cytochrome oxidase subunit IV; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; CL, bovine cardiolipin; POPE, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine; octyl-POE, octylpolyoxyethylene

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analyzed by CD and fluorescence spectroscopy. The results show that the fusion protein bound to phospholipid vesicles takes a significantly altered conformation, but is not completely unfolded.

2. MATERIALS AND METHODS

The fusion protein pCox IV-DHFR was purified from an *Escherichia coli* strain harboring the expression plasmid pKK-pCox IV-DHFR as described previously [3]. Phospholipid vesicles were prepared from a mixture of POPC, CL and POPE by reverse-phase evaporation as described previously [3]. Lipids were from Avanti Polar Lipids, Inc. (USA).

CD spectra were recorded on a Jasco J-20A spectropolarimeter and fluorescence spectra on a Hitachi F-3000 fluorescence spectrophotometer at 25°C.

3. RESULTS

The effects of phospholipid vesicles (POPC/CL/POPE = 7:3:0.35) on the CD spectra of the fusion protein are shown in fig.1A. The addition of a vesicle suspension increased the ellipticity at <230 nm and shifted the CD minimum from 208 nm to around 225 nm. This change in the CD spectrum parallels the change in the protein's tryptophanyl fluorescence emission spectrum, which is red-shifted on addition of vesicles ([3], fig.2). Both CD and fluorescence spectra change with increasing concentrations of lipids up to 15 μM of phosphate, giving a peptide to lipid stoichiometry of about 1:15 in the bound state.

It is important to estimate the contribution of the presequence conformational change to the observed CD spectral change, since this region is known to adopt an α-helical structure upon binding to surfaces [10]. However, at identical concentrations, the difference in CD spectra of the synthetic presequence peptide p25 with and without SDS micelles is small as compared to the change in CD spectra of the fusion protein (fig.1B). Most of the change in the CD spectrum of the fusion protein is, therefore, due to the alteration in the secondary structure of the DHFR moiety. This vesicle-induced spectral change does not correspond to total unfolding, since the CD spectrum of the fusion protein in 5 M urea (fig.1B) does not resemble that with >15 μM lipid vesicles

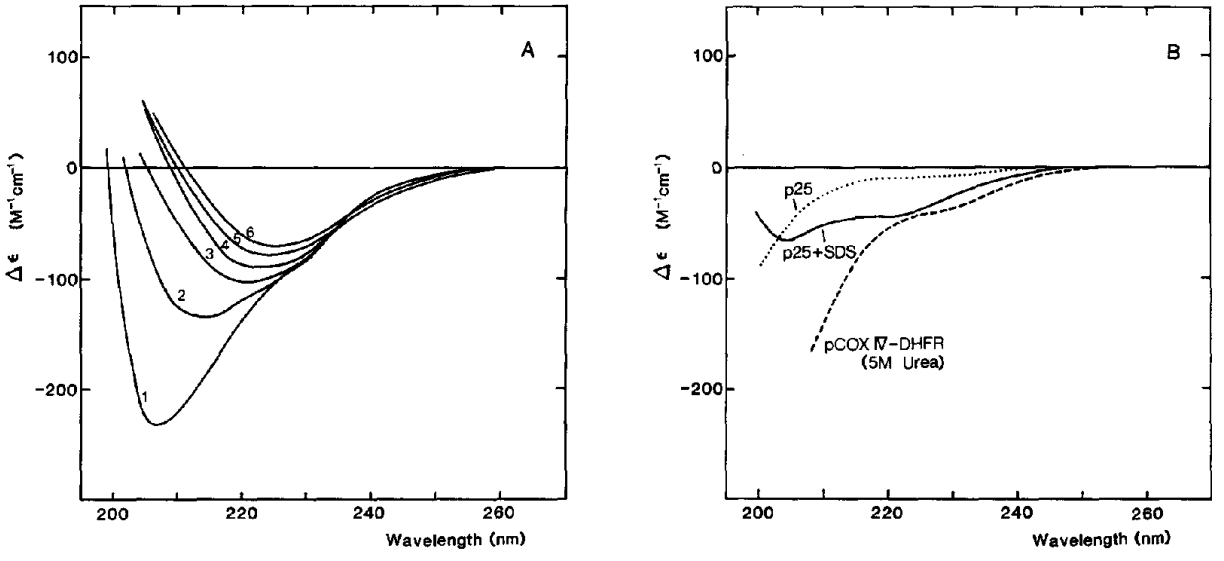

Fig.1. (A) CD spectra of pCox IV-DHFR (1 μM) in 20 mM Tris-Cl (pH 7.0) and 50 mM KCl at 25°C in the presence of various concentrations of phospholipid vesicles (POPC/CL/POPE = 7:3:0.35). Curves 1–6 indicate 0, 4, 8, 12, 16 and 24 μM of lipids, respectively. (B) CD spectra of p25 (1 μM) in the same buffer as (A) with (——) and without (···) 5 mM of SDS, and a CD spectrum of pCox IV-DHFR (1 μM) in the same buffer as (A) including 5 M urea (– – –). Although methanol and a neutral detergent lysophosphatidylcholine cause a CD spectral change of p25 similar to SDS, effects of phospholipid vesicles on the CD spectra of p25 are difficult to follow because of the extensive aggregation (Roise, D., personal communication).

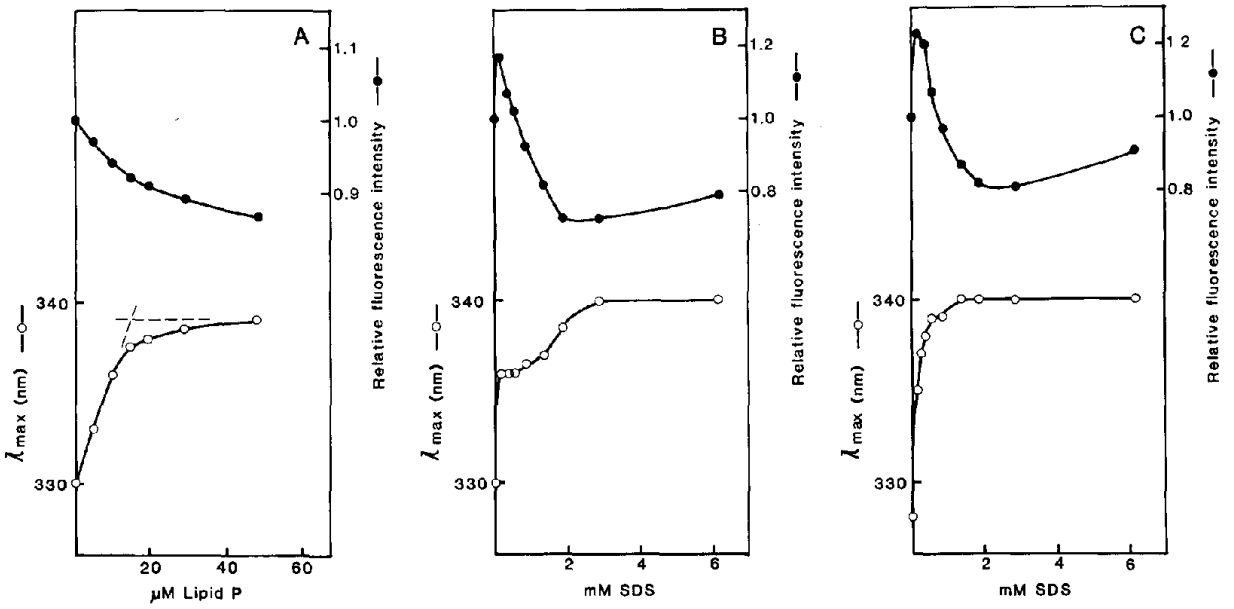

Fig.2. Emission maximum and intensity of tryptophanyl fluorescence (excited at 280 nm) as a function of (A) concentrations of lipids of vesicles (POPC/CL/POPE = 7:3:0.35) for pCox IV-DHFR, (B) concentrations of SDS for pCox IV-DHFR, and (C) concentrations of SDS for mouse DHFR at 25°C. Buffers contain 20 mM Tris-Cl (pH 7.0) and 50 mM KCl (A) or 20 mM Tris-Cl (pH 7.0) (B and C).

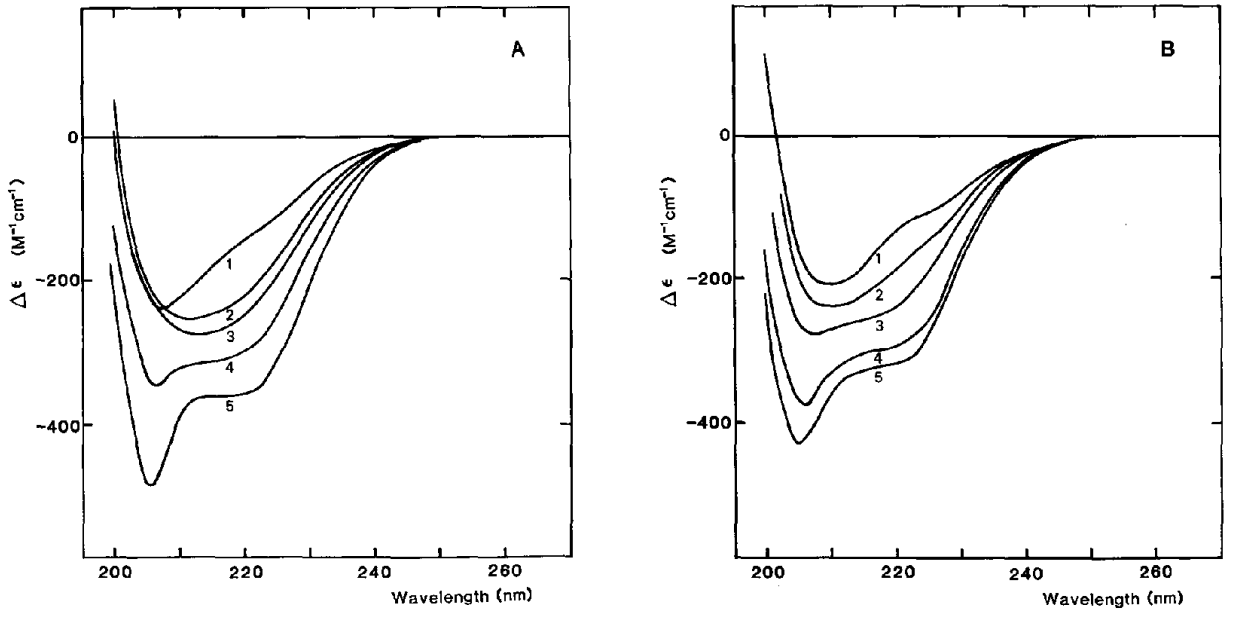

Fig.3. CD spectra of (A) pCox IV-DHFR and (B) mouse DHFR in Tris-Cl (pH 7.0) at 25°C in the presence of various concentrations of SDS. Curves 1–5 indicate 0, 0.17, 0.51, 1.4 and 2.9 mM SDS, respectively. Critical micelle concentration of SDS under the present concentration is about 1 mM.

(fig.1A). (The fusion protein is completely denatured in 5 M urea [3].) The CD spectrum of mouse DHFR changes little in the presence of even 42 μM lipid vesicles (POPC/CL/POPE = 70:30:3.5; data not shown), which is consistent with the fact that mouse DHFR without a presequence cannot interact with lipid vesicles [3].

SDS is often used as an analog of acidic phospholipids to probe protein-lipid interactions. Thus we also examined the effects of SDS on CD and fluorescence spectra of the fusion protein and mouse DHFR (figs 2 and 3). The conformational changes caused by the binding of SDS appear to consist of two processes. First, the presence of 0.1–0.2 mM SDS induces a negative CD band at around 210–220 nm, reflecting increase in a helical fraction, and causes a red-shift of tryptophanyl fluorescence by 6 nm. Further addition of SDS to 1–3 mM increases a negative CD band at around 205 nm, reflecting increase in a random coil fraction, and causes an additional red-shift of tryptophanyl fluorescence by 4–6 nm. In contrast to phospholipid vesicles, SDS micelles induced essentially the same CD and fluorescence spectral changes for mouse DHFR as for the fusion protein. The higher concentration of SDS required to change the fusion protein's conformation is probably due to the presence of the additional SDS-binding sites in the presequence. The SDS-induced conformational change is, therefore, not presequence-dependent as observed for phospholipid vesicle binding.

4. DISCUSSION

Previous studies have shown that binding of the fusion protein to acidic phospholipid-containing vesicles involves at least partial unfolding of the fusion protein [3,4], but this structure has not yet been well defined. For example, since hydrophobic lipid environments may affect the fluorescence properties, it is not clear whether the red-shift of only 8–9 nm on addition of phospholipid vesicles ([3], fig.1), as compared with that of about 20 nm on overall protein denaturation, reflects partial unfolding or extensive unfolding.

The present study employing CD and fluorescence spectroscopy has revealed that binding of the fusion protein to phospholipid vesicles accompanies a significant change in the folding of the DHFR moiety. The vesicle-bound conformation appears to be labile (from a previous study [4]) but distinct from a random coil (from CD spectra), although detailed analysis of the CD spectral change is difficult because of unpredictable contribution of the turbidity increase. Since presequence-free mouse DHFR does not exhibit such a CD spectral change as observed for the fusion protein in the presence of vesicles, the conformational change crucially requires the interaction between the presequence and (acidic) phospholipids. It is interesting to note that the CD spectra of pCox IV-DHFR bound to lipid vesicles and to SDS micelles closely resemble those of apocytochrome *c*, the import-competent form of cytochrome *c* (without a cleavable presequence); helical structures are induced in apocytochrome *c* upon binding to lipid vesicles or SDS micelles [11,12].

The fact that phospholipid vesicles, but not SDS micelles, can induce a presequence-dependent conformational change may reflect a difference in the monomeric concentrations of SDS and phospholipids. Even above the critical micelle concentration, the monomeric SDS concentration is high enough, as compared with phospholipids, to cause further denaturation of the mature part of the fusion protein: this SDS denaturation would mask the presequence-dependent conformational change of the fusion protein, if any. Instead, the difference in the organization of lipids such as micelles and a bilayer phase may be responsible for the difference in the fusion protein's conformational change. The latter interpretation is consistent with the previous observation that the vesicle-bound fusion protein solubilized with a neutral detergent octyl-POE retains a trypsin-sensitive conformation while mixed micelles composed of phospholipids (including acidic phospholipids) and octyl-POE do not in turn induce a trypsin-sensitive conformation [4]. In this case, the presence of a distinct and stable lipid-water interface provided by a bilayer phase may cause a kind of irreversible surface denaturation of the precursor protein.

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