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Functional Characterization of the Conserved "GLK" Motif in Mitochondrial Porin from *Neurospora crassa*

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Mitochondrial porin facilitates the diffusion of small hydrophilic molecules across the mitochondrial outer membrane. Despite low sequence similarity among porins from different species, a "glycine–leucine–lysine" (GLK) motif is conserved in mitochondrial and *Neisseria* porins. To investigate the possible roles of these conserved residues, including their hypothesized participation in ATP binding by the protein, we replaced the lysine residue of the GLK motif of *Neurospora crassa* porin with glutamic acid through site-directed mutagenesis of the corresponding gene. Although the pores formed by this protein have size and gating characteristics similar to those of the wild-type protein, the channels formed by GLEporin are less anion selective than the wild-type pores. The GLEporin retains the ability to be cross linked to [α-^{32}P]ATP, indicating that the GLK sequence is not essential for ATP binding. Furthermore, the pores formed by both GLEporin and the wild-type protein become more cation selective in the presence of ATP. Taken together, these results support structural models that place the GLK motif in a part of the ion-selective β-barrel that is not directly involved in ATP binding.

KEY WORDS: Mitochondrial porin; VDAC; ATP binding; *Neurospora crassa*.

INTRODUCTION

Mitochondrial porin, a voltage-dependent anion-selective channel (VDAC), spans the outer membrane of the organelle, where it is responsible for the transport of small molecules between the cytosol and the intermembrane space (Zalman *et al.*, 1980; Roos *et al.*, 1982; Schein *et al.*, 1976; Colombini, 1979). Mitochondrial porin forms a water-filled channel, which, when inserted into artificial lipid bilayers, is slightly anion selective in its high-conductance open states (4.0–4.5 nS in 1 M KCl) and cation selective in the voltage-induced, lower-conductance, partially "closed" states (2.0–2.5 nS) (reviewed in Benz, 1994). The physiological importance of this partial closure, or gating, is suggested by its modulation by compounds including NADH (Zizi *et al.*, 1994) and intermembrane-space proteins (Holden and Colombini, 1993).

The DNA sequences encoding mitochondrial porins from a number of species are known at present (Mihara and Sato 1985; Kleene *et al.*, 1987; Troll *et al.*, 1992; Heins *et al.*, 1994; Kayser *et al.*, 1989; Fischer *et al.*, 1994; Blachly-Dyson *et al.*, 1993, 1994, 1997; Ha *et al.*, 1993; Sampson *et al.*, 1996a, b; Elkeles *et al.*, 1995; Bureau *et al.*, 1992). Although the overall identity in the derived protein sequences is relatively low, channel properties, such as voltage dependence, ion selectivity, and single-channel conductance are highly conserved. In addition, the proteins are predicted to possess similar secondary structures, containing stretches of alternating hydrophobic and hydrophilic amino acids that suggest the formation of amphipathic β-strands (Benz, 1994; de Pinto *et al.*, 1991; Blachly-Dyson *et al.*, 1990). Several structural models for mitochondrial porins predict β-barrel cylinders consisting of 16 antiparallel, amphiphilic β-

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strands (Benz, 1994; de Pinto *et al.*, 1991) like those of the bacterial porins (Cowan *et al.*, 1992). Other models are based on a pore formed by the amphiphilic N-terminal α-helix and either 12 (Blachly-Dyson *et al.*, 1990) or 13 β-strands (Song *et al.*, 1998). Circular dichroism (CD) studies of porin purified from mitochondria (Shao *et al.* 1996) or expressed in and purified from *Escherichia coli* (Popp *et al.*, 1997; Koppel *et al.*, 1998) confirm that this porin has a high β-sheet content, as predicted from its primary sequence.

Despite the low overall sequence similarity, a glycine–leucine–lysine (GLK) motif is highly conserved among mitochondrial porins from different species and in porins from *Neisseria* (Troll *et al.*, 1992; Fig. 1). It has been proposed that these residues are involved in the binding of the purine nucleotides ATP and GTP because the motif resembles the GTP binding site in the T-cell receptor ζ chain (Rudel *et al.*, 1996). In the latter protein, the lysine residue in a glycine–methionine–lysine (GMK) motif was found to be the site to which GTP could be chemically cross linked (Peter *et al.*, 1992). Here we test the role of the GLK motif in ATP binding by the mitochondrial porin of *Neurospora* by creating a mutant version of the protein in which the GLK sequence is replaced by glycine–leucine–glutamate (GLE). The electrophysiological and structural properties of the resulting GLEporin are also investigated and compared to those of the wild-type protein.

Organism/Porin Swiss-Prot		Sequence
Neurospora crassa Porin1 P07144	83	KVEMADNLAK**GLK**AEGIFSFLPA
Saccharomyces cerevisiae Porin1 P04840	83	KLEFAN-LTP**GLK**NELITSLTPG
Saccharomyces cerevisiae Porin2 P40478	82	RIEFSK-IAP**GWK**GDVNAFLTPQ
Solanum tuberosum (Potato) POM34 P42055	82	TITVDE-AAP**GLK**TILS-FRVPD
Solanum tuberosum (Potato) POM36 P42056	95	TITVDE-PAP**GLK**TIFS-FVVPD
Triticum aestivum (Wheat) Porin1 P46274	95	TITADDLAAP**GLK**TILS-FAVPD
Dictyostelium discoideum Porin1 Q01501	109	EFTIEN-IIP**GLK**AVAN----GD
Xenopus laevis Porin2 P81004	82	EIAIEDQIAK**GLK**LTFDTTFSPN
Mus musculus (Mouse) Porin1 Q60932	83	EITVEDQLAR**GLK**LTFDSSFSPN
Mus musculus (Mouse) Porin2 Q60930	78	EIAIEDQICQ**GLK**LTFDTTFSPN
Mus musculus (Mouse) Porin3 Q60931	78	EISWENKLAE**GLK**LTLDTIFVPN
Rattus norvegicus (Rat) Porin2 P81155	78	EIAIEDQICQ**GLK**LTFDTTFSPN
Homo sapiens Porin1 P21796	82	EITVEDQLAR**GLK**LTFDSSFSPN
Homo sapiens Porin2 P45880	81	EIAIEDQICQ**GLK**LTFDTTFSPN
Neisseria gonorrhoeae PorB P18195	99	TGWGNKQSFV**GLK**GGFGTIRAGS
Neisseria meningitidis PorB P30687	99	SGWGNRQSFI**GLK**GGFGKLRVGR

Fig. 1. The conserved GLK motif. The amino acid sequences of the indicated porins were aligned using the Clustal 1.7 software (Thompson *et al.*, 1994); a short sequence including the conserved GLK (bold text) is presented here. The number to the left of the sequence alignment indicates the position of the adjacent residue in the corresponding protein sequence.

MATERIALS AND METHODS

Strains

Escherichia coli strains CJ236 (Kunkel *et al.*, 1987) and DH5α (Hanahan, 1983) were utilized in gene construction; recombinant porin proteins were purified from strains XL1-Blue (Bullock *et al.*, 1987) or M15 (Villarejo and Zabin, 1974).

Site-Directed Mutagenesis

The porin constructs used in this study were derived from the cDNA clone obtained by Kleene *et al.* (1987). The cDNA was recloned into a pBluescript SK^- vector (Stratagene, La Jolla, CA) and the resulting construct used for the generation of single-stranded DNA to be used for site-directed mutagenesis (Kunkel *et al.*, 1987). The T7 mutagenesis kit (BioRad, Mississauga, ON, Canada) and an oligonucleotide obtained from Life Technologies (Burlington, ON, Canada) were used to create a porin-coding sequence in which the lysine residue at position 95 was replaced with a glutamate residue. The mutagenic oligonucleotide (5′-CAAGGGTCTCGAGGCTGAGGGTATC) was used to introduce a base change of A to G (underlined) at base 327 in the sense strand to produce plasmid pBST–GLEporin. This change also introduced a new *Xho*I recognition site. The sequence of the mutated region and flanking DNA to be used in further constructions was confirmed by DNA sequencing at the DNA sequencing facility in the Institute of Cell Biology, University of Manitoba.

To clone the mutant coding sequence into the expression vector pQE-9 (Qiagen, Hilden, Germany), we first PCR-amplified a 320-bp fragment encompassing the mutation from pBST-GLEporin. The upstream primer (5′-AAAGGATCCATGGCTGTT CCCGCTTTC) incorporates a *Bam*HI site (underlined) at the 5′-end of the PCR product and the second primer (5′-CTTGAAGTGGAGGTTGAACTTGGC) hybrid-

izes to a sequence downstream of the endogenous *Nar*I site. The PCR product was then digested with *Bam*HI and *Nar*I and the resultant 293-bp fragment was used to replace the corresponding fragment in the pQE-9 derived plasmid that encodes the wild-type porin, His_6porin (Popp *et al.*, 1996). The resulting plasmid encodes GLEporin, which includes an N-terminal hexahistidinyl tag. The sequence of the *Bam*HI-*Nar*I fragment was confirmed by DNA sequencing.

Protein Expression and Purification

Overnight cultures in LB (Sambrook *et al.*, 1989) or DYT (Popp *et al.*, 1996) medium of *E. coli* M15 or XL1-Blue containing the pQE-9 plasmids encoding His_6porin or GLEporin were grown to an A_{600} of 0.7 to 0.9. Protein expression and purification by affinity chromatography on Ni–NTA agarose were carried out as previously described (Popp *et al.*, 1996) and protein purity was monitored by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). The purified protein samples used for biophysical analysis and cross linking studies were diluted 1:10 in 1% Genapol X-80 (Fluka, Buchs, Switzerland), 1 mM EDTA, 10 mM potassium phosphate, and dialyzed extensively against the same buffer. The purified protein used for cross-linking studies was diluted 1:2 in 1% Genapol X-80, 10 mM potassium phosphate, pH 7, and dialyzed against the same buffer. Protein used for circular dichroism (CD) measurements was diluted 1:2 into 1% Genapol X-80, 10 mM potassium phosphate, pH 7, and concentrated in a Centricon 10 column (Amicon, Inc., Beverly, MA) before extensive dialysis against the same buffer.

Cross Linking of Purified Porin to [α^{32}P]ATP through Periodate Oxidation

Approximately 100 ng of purified porin were diluted in phosphate-buffered saline (PBS) containing 10 mM $MgCl_2$ to a volume of 100 μL and incubated with 10 μCi of [α-^{32}P]ATP (3000 Ci/mmol; New England Nuclear, Boston, Massachusetts) for 10 min at room temperature. Samples were then incubated in the presence or absence of cross-linking reagents as previously outlined (Rudel *et al.*, 1996). Subsequently, proteins were precipitated with nine volumes of ice-cold acetone, washed twice with 70% acetone, resuspended in loading buffer, and separated by SDS–PAGE (Sambrook *et al.*, 1989). Gels were stained with Coomassie Brilliant blue and then dried. The dried gels were exposed to Kodak (X-OMAT) film for 10 days.

Lipid Bilayer Experiments

The methods used for the black-lipid bilayer experiments have been previously described (Benz *et al.*, 1978). In short, membranes were made by application of a 1% (w/v) solution of diphytanoyl phosphatidylcholine (DiphPC, Avanti Polar Lipids, Alabaster, Alabama) in *n*-decane across circular holes with a surface area about 0.1 mm^2 in the thin wall of a Teflon cell, which separates two 5-mL aqueous compartments. The aqueous solutions were either buffered with 10 mM *N*-[2-hydroxylethyl]piperazine *N*-[2-ethanesulfonic acid] (HEPES) to pH 7 or unbuffered at pH 6. Dialyzed protein solution, containing 0.5% (w/v) ergosterol, was added to the aqueous phase in the *cis* compartment where the voltage was applied after the membranes had turned optically black in reflected light. Zero-current membrane potential measurements were performed by establishing a salt gradient across membranes containing 100–1000 porin channels, as described previously (Benz *et al.*, 1979).

Circular Dichroism

CD spectra were obtained using a JASCO J-500A spectropolarimeter calibrated with (+)-10-camphorsulfonic acid. The instrument was purged with N_2 at 5 L/min above 210 nm and at 25 L/min below 210 nm. Spectra were measured, in a quartz cell with a 0.5-mm path length, at a scan rate of 0.5 nm/min and with a time constant of 32 s. Sample temperature was maintained with the use of a circulating water bath. The concentrations of His_6porin and GLEporin samples were determined from their molar extinction coefficients and absorbance at 280 nm.

The secondary structures of His_6porin and GLEporin were determined using the convex constraint algorithm (CCA) of Perczel *et al.* (1992). The CD spectra of His_6porin and GLEporin were individually appended to the membrane-protein reference data set of Park *et al.* (1992) for deconvolution by CCA.

RESULTS

Properties of Single Channels Formed by GLEporin

To study the functional properties of porin protein in which the conserved GLK motif (Fig. 1) was altered, we generated a version of the *Neurospora* porin in which this conserved sequence was replaced by glycine–leucine–glutamate (GLE). For purification from *E. coli*, the mutant porin was expressed with an amino-terminal hexahistidinyl tag, the presence of which does not alter the electrophysiological properties of the pore (Popp *et al.*, 1996; Koppel *et al.*, 1998). The single-channel recordings generated from GLEporin (Fig. 2A) are similar to those of His_6porin (Popp *et al.*, 1996); the conductance increase produced by both proteins occurs in distinct steps of similar amplitudes. The majority of the conductance steps were between 4 and 5 nS for GLEporin (Fig. 2B), similar to those of His_6porin (Popp *et al.*, 1996). The voltage-dependent gating of the channels formed by the GLEporin mutant was also examined (Fig. 3) and was found to be similar to that of His_6porin at applied voltages of greater than 30 mV; for example, the G_U/G_O of GLEporin at 50 mV was 0.52 compared to 0.43 for His_6porin (Popp *et al.*, 1996). However, the GLEporin appeared to be slightly less sensitive to gating at very low negative voltages (−20 mV).

The GLEporin channels are cation-selective (P_C/P_A of 1.6 in 0.05 M KCl; Fig. 4), in contrast to the anion-selective native wild-type porin and His_6porin, for which the P_C/P_A values are 0.77 and 0.67, respectively (Popp *et al.*, 1996).

We also examined the effect of ATP on ion selectivity; binding of this negatively-charged molecule to the inside of the pore would likely have an effect on the charge distribution of the lining of the pore and, hence, its ion selectivity. A tenfold KCl gradient was established across a porin-containing black-lipid membrane starting from 0.05 M KCl; buffered ATP was added, to a maximum concentration of 5 mM, to the chamber containing the dilute KCl solution. Zero-current membrane potential was measured and used to determine ion selectivity. Figure 4 shows the effects of increasing ATP concentration on the ion selectivity of wild type and GLEporin. In the presence of ATP, P_C/P_A for His_6porin was 3.5, compared to 0.6 for this porin exposed to the same gradient in the absence of ATP. An increase in cation selectivity was also observed for GLEporin. The presence of ATP increased P_C/P_A from 1.6 to 5. These results suggest that ATP binding has a similar net effect on the ion selectivity of both wild type and GLEporin.

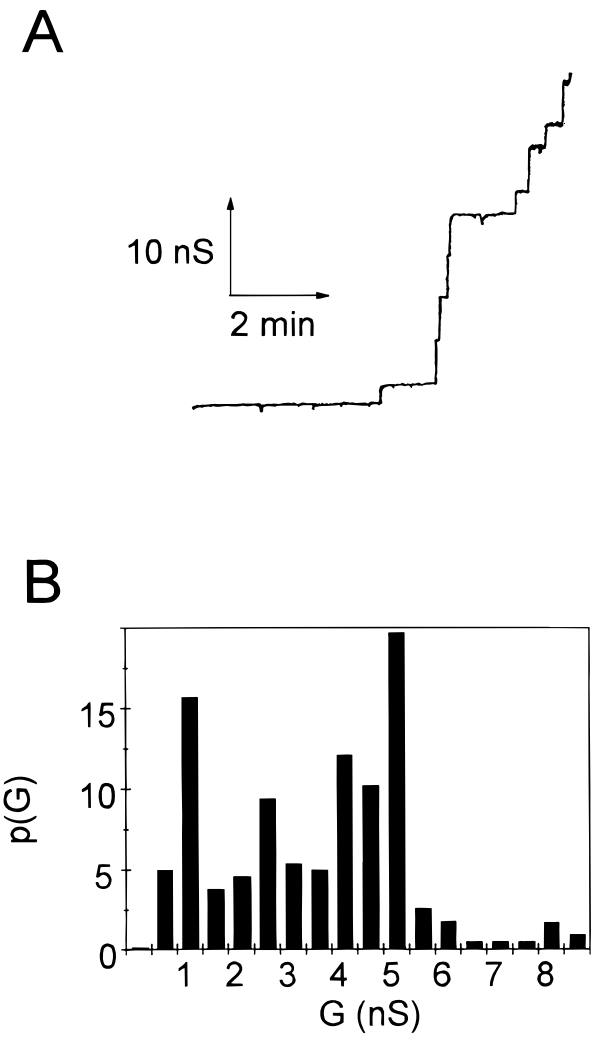

Fig. 2. (A) Single-channel recording of GLEporin inserted into diphytanoyl phosphatidylcholine/*n*-decane membranes. GLEporin (2.5 μL) (2–30 ng/μl with 0.5% ergosterol in 1% Genapol X-80 buffer) were added to the *cis* compartment. Addition of the protein took place before the recording. (B) Histogram of the conductance fluctuations of GLEporin inserted into black-lipid bilayers. p(G) is the frequency that a given conductance increment G is observed in the single-channel experiment. p(G) was calculated by dividing the number of fluctuations with a given conductance increment by the total number of conductance fluctuations. The aqueous phase contained 1 M KCl (pH 6.0). The applied membrane potential was +10 mV; T = 20°C. The number of observed conductance increments was 251.

ATP Binding by GLEporin

Chemical cross linking utilizing purified porins and [α-^{32}P]ATP was used to confirm that GLEporin,

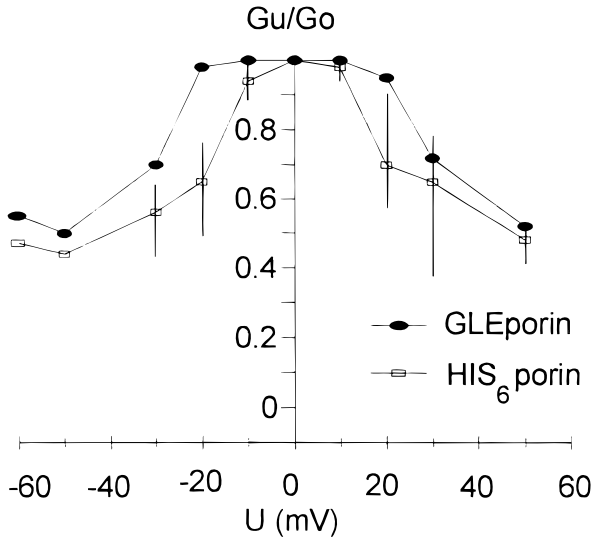

Fig. 3. Voltage dependence of His_6porin and GLEporin. The ratio of the conductance, G_u, at a given voltage, U, divided by G_o at 10 mV is shown as a function of voltage. Experiments were repeated between two and four times; the average G_u/G_o values are plotted. The data for His_6porin (□) are replotted from (Popp *et al.*, 1996); vertical lines indicate the standard deviation of the data points. Standard deviations for the GLEporin data (●) were all less than 3% of the plotted data and, therefore, too small to be indicated on the plot. The membranes were formed of DiphPC/*n*-decane. The aqueous phase contained 1 M KCl (pH 6.0); T = 20°C.

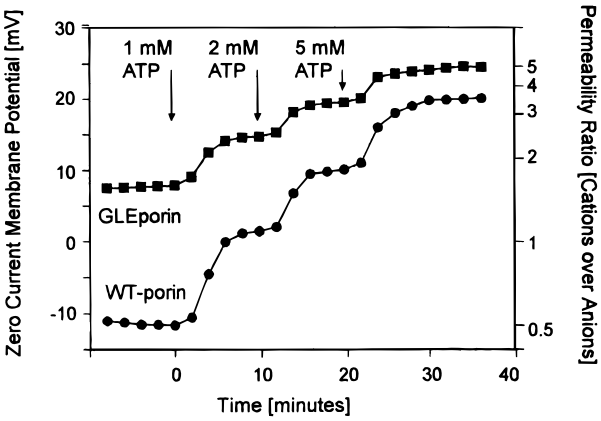

Fig. 4. Effect of increasing concentrations of ATP on the selectivity of wild-type mitochondrial porin from *Neurospora crassa* (closed circles) and on that of the GLE mutant porin (closed squares). A tenfold KCl gradient (50 vs 500 mM) was established across diphytanoyl phosphatidylcholine/*n*-decane membranes in which about 200 porin channels were reconstituted. The zero-current membrane potentials were measured at the more dilute side (left axis). When the potential was in equilibrium, ATP was added to both sides of the membrane (arrows) at the concentrations indicated at the top of the panel. The equilibrium zero-current membrane potential was reached approximately 8 min after the addition of ATP. The right axis shows the permeability ratio P_C/P_A.

like the wild-type protein, is capable of ATP binding. In these experiments (Fig. 5), purified His_6porin was covalently labeled in reactions containing cross-linking reagents, indicating that His_6porin binds ATP similarly to the native protein and that the N-terminal His_6 tag does not interfere with this binding. GLEporin also bound ATP, indicating that the conserved GLK motif is not essential for ATP binding by *N. crassa* mitochondrial porin.

Circular Dichroism

The porin proteins used in this study were eluted from the Ni–NTA columns in the presence of 8 M urea, which denatures porin (Popp *et al.*, 1997). Therefore, prior to circular dichroism analyses, purified His_6porin and GLEporin were transferred to a buffer containing Genapol X-80 by dialysis.

The CD spectra of His_6porin and GLEporin are presented in Fig. 6A. Spectra of His_6porin and GLEporin show broad minima with negative peaks at 215 and 218 nm, respectively (Table I). The wavelengths at which the molar ellipticities are zero ($\lambda_{crossover}$) are also similar for the two proteins: 207.5 nm for His_6porin and 209.5 nm for GLEporin. The CD spectra of His_6porin and GLEporin were further analyzed to determine the relative contributions of pure structural components to the spectra. The CCA algorithm by Perczel *et al.* (1992) was used and data for each porin were appended individually to the membrane protein data set of Park *et al.* (1992). Initially, the number of pure component curves (P) comprising the data sets

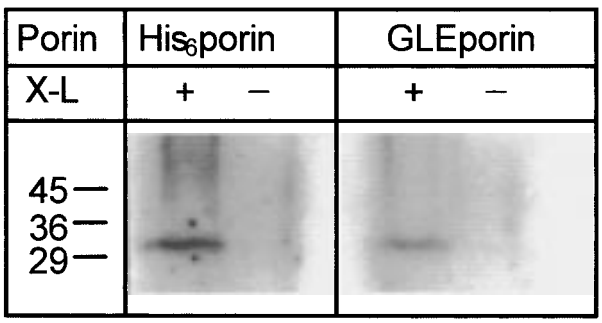

Fig. 5. Covalent coupling of [α-^{32}P]ATP to His_6porin and GLEporin. His_6porin and GLEporin were incubated with [α-^{32}P]ATP and chemically cross linked (+ X-L). As a negative control, His_6porin and GLE porin were incubated with [α-^{32}P]ATP in the absence of cross linker (− X-L). Samples were analyzed by SDS–PAGE; autoradiographs of the dried gels are presented. Numbers to the left of the figure indicate the positions of molecular weight markers run on the same gel (kDa).

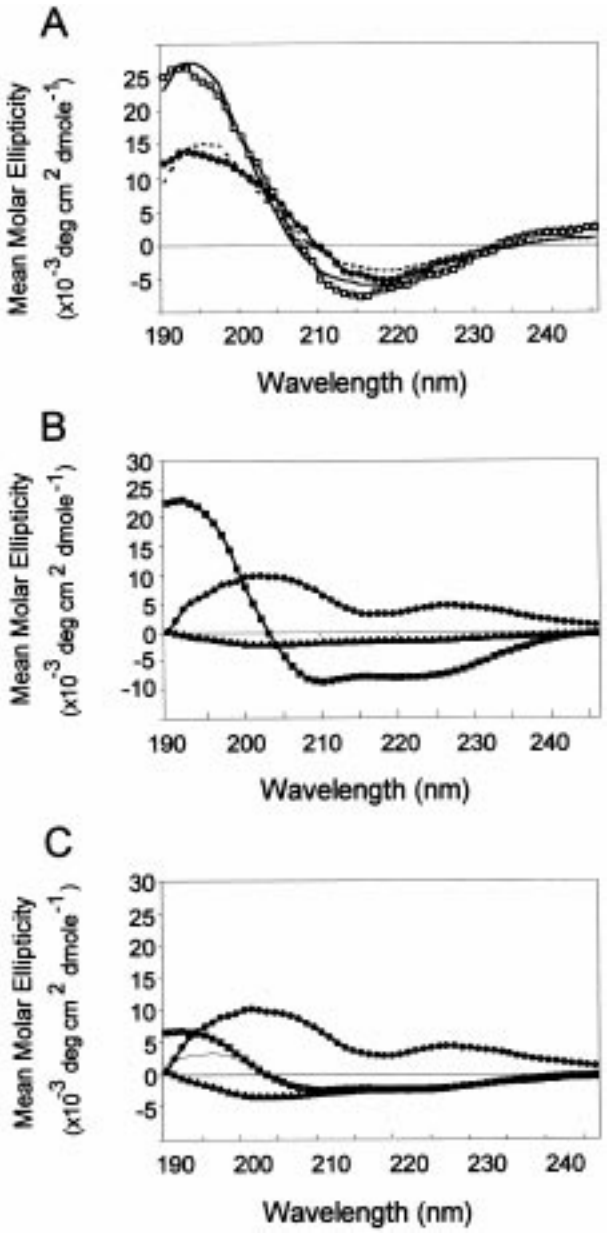

Fig. 6. Circular dichroism analyses of His_6porin and GLEporin. (A) The experimentally measured data for His_6porin (0.027 mg/ml) and GLEporin (0.066 mg/ml) (□ and ○, respectively) and those calculated from the weighted pure component curves (solid and dashed lines, respectively) produced by convex constraint algorithm (CCA) analysis (Perczel *et al.*, 1992) are presented. Pure component curves generated by CCA analysis of His_6porin (B) and GLEporin (C) are also presented; the curves are weighted based on the fraction of each component. □, α-helix; ○, β-sheet; Δ, random coil; solid line, α_t-helix. Note that the spectrum for wild-type porin does not contain α_t-structure and, therefore, there is not a curve representing this component in (B).

were determined *a priori*. We analyzed both sets of data, using 2, 3, 4, or 5 for the value of *P.* When $P = 2$, 3, or 5, one or more of the pure component curves was unidentifiable for both proteins. For $P = 4$, however, all of the pure curves correspond to the spectra arising from pure secondary structures (Perczel *et al.*, 1992). Figures 6B and C show the pure component curves generated for His_6porin and GLEporin for $P = 4$. In this case, the curves correspond to α-helix, transmembrane α-helix (α_t helix), β-sheet, and random coil. The estimated amounts of these four structures for His_6porin are 31% α-helix, 0% α_t helix, 47% β-sheet, and 22% random coil (Table I). For GLEporin (Fig. 6C and Table I), the estimate of β-sheet content is similar, 47%, and the amount of transmembrane α-helix is also very low (6%). However, the α-helical content in GLEporin is lower (9%) than in His_6porin (31%); this decrease is accompanied by a comparable increase in random coil structure to 38%.

DISCUSSION

Single-Channel Conductance, Voltage Gating, and Ion Selectivity of the GLE Mutant

The GLEporin forms stable open channels that display normal voltage-dependent gating, but are more cation selective than the wild-type His_6porin. A similar change in ion selectivity was reported for the equivalent GLEporin of *Saccharomyces* (Blachly-Dyson *et al.*, 1990). The altered ion selectivity suggests that the GLK motif is located within a β strand that forms the pore lining, where it would contribute to the net charge of the water-exposed surface inside the pore. This location of the GLK motif is in agreement with the current structural models of *N. crassa* mitochondrial porin (Benz, 1994; de Pinto *et al.*, 1991; Popp, 1996) and *Neisseria* PorB (Derrick *et al.*, 1999), which position this motif in the β-barrel of the protein, near the junction of the barrel and a membrane-exposed loop. However, without X-ray crystallographic data, it cannot be ruled out that this motif is located in a cytoplasmically exposed loop that contributes to ion selectivity through a long-range electrostatic effect.

Interactions of GLEporin with ATP

The experiments presented herein demonstrate that the His_6porin (Fig. 5), like native porin purified

Table I Compilation of Circular Dichroism Data Obtained from *Neurospora* Porins

Porin	Conditions	$\lambda_{crossover}$ (nm)	λ_{min} (nm)	α-Helical content (%)	β-Strand content (%)	Other (%)	Reference
His_6porin	1% Genapol X-80, pH 7	207.5	215	31[a]	47	22[b]	This work
GLEporin	1% Genapol X-80, pH 7	209.5	218	15[a]	47	38[b]	This work
His_6porin	1% LDAO,[c] pH 8	205.0	216	12	45	37[d]	Koppel *et al.*, 1998
Native porin	1% LDAO, pH 8	205.0	214	12	45	37[d]	Koppel *et al.*, 1998
Native porin	2% β-OG,[e] pH7	205.5	216	30	62	8[d]	Shao *et al.*, 1996

[a] Includes transmembrane α-helix (α_t).
[b] Random coil.
[c] Lauryl dimethylamine oxide.
[d] β-turn plus random coil.
[e] β-Octylglucoside.

from mitochondria (Rudel *et al.*, 1996), binds ATP, which can be subsequently cross linked to the protein via periodate oxidation. Thus, the hexahistidinyl tag, engineered onto the N-terminus of the protein for purification purposes, does not interfere with ATP binding or the subsequent cross-linking reaction. GLEporin was also cross linked to ATP, indicating that the replacement of the lysine residue by glutamate does not disrupt ATP binding to mitochondrial porin. This conclusion is supported by the observation that GLEporin, like the wild-type molecule, becomes more cation selective in the presence of ATP, as expected if a negatively-charged molecule is bound either within the channel, or in the vicinity of the opening of the pore. Taken together, these results suggest that, although the GLK motif resembles the GMK motif in the T cell receptor ζ chain that is important for GTP binding (Peter *et al.*, 1992), it is not part of the ATP-binding site of mitochondrial porin.

Circular Dichroism

As summarized in Table I, the CD spectrum of wild-type His_6porin in Genapol X-80 is very similar to the spectra observed by Shao *et al.* (1996) for native *Neurospora* porin in 2% β-octylglucoside (β-OG), pH 7, and in lauryl dimethylamine oxide (LDAO), pH 8 (Koppel *et al.*, 1998), and for recombinant pea root plastid porin in Genapol X-80 (Popp *et al.*, 1997). The CD results are also similar to those of His_6porin created by Koppel *et al.* (1998) and examined at pH 8 in LDAO (Table I). Thus, mitochondrial porin maintains high β-content in a variety of detergents between pH 7 and 8. Furthermore, the addition of 12 (this work) or 20 (Koppel *et al.*, 1998) N-terminal amino acid residues, including the hexahistidinyl tag, does not alter this characteristic. Although these results support models for mitochondrial porin, based on a β-barrel structure, the lack of information concerning the length of each β-strand does not allow the calculated β-sheet content to be used as a criterion for excluding or supporting the models for porin currently presented in the literature (see Introduction).

Deconvolution of the CD spectrum of the mutant GLEporin also indicates that the β-strand content of this protein, in detergent, is identical to that of the native porin (Table I). Thus, the detergent appears to promote the folding of similar β-strand structures in the wild-type and mutant proteins, an observation in agreement with the similar electrophysiological properties of the two proteins. However, there are small differences in the amounts of total α-helix and random coil structure between His_6porin (31 and 22%, respectively) and GLEporin (15 and 38%), which are not reflected in the behavior of GLEporin in black-lipid bilayers. Presumably these structural differences affect the exposed loops of the protein, and if so, the results may indicate that these parts of the GLEporin molecule fold slightly differently in detergent and in lipid membranes. It is important to consider, however, that the GLEporin forms "normal" pores in black-lipid bilayers. Presumably, these structural differences affect the exposed loops of the protein, and if so, these results may indicate that detergent and lipid membranes promote slightly different folding of these parts of the molecule in the GLEporin.

In summary, the GLK motif is not essential for pore formation or ATP binding by mitochondrial porins. The lysine residue does contribute to the anion-selective nature of the pore, suggesting a possible reason for conservation of the motif in many mitochondrial porins.

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