Cloning and Functional Expression in Yeast of Two Human Isoforms of the Outer Mitochondrial Membrane Channel, the Voltage-dependent Anion Channel*

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The voltage-dependent anion channel (VDAC) of the outer mitochondrial membrane is a small abundant protein found in all eukaryotic kingdoms which forms a voltage-gated pore when incorporated into planar lipid bilayers. VDAC is also the site of binding of the metabolic enzymes hexokinase and glycerol kinase to the mitochondrion in what may be a significant metabolic regulatory interaction. Recently, there has been speculation that there may be multiple forms of VDAC in mammals which differ in their localization in the outer mitochondrial membrane and in their physiological function. In this report, we describe the identification and characterization of two human cDNAs encoding VDAC homologs (HVDAC1 and HVDAC2). To confirm VDAC function, each human protein has been expressed in yeast lacking the endogenous VDAC gene. Human proteins isolated from yeast mitochondria formed channels with the characteristics expected of VDAC when incorporated into planar lipid bilayers. In addition, expression of the human proteins in such strains can complement phenotypic defects associated with elimination of the endogenous yeast VDAC gene. Since VDAC is the site of binding of hexokinase to the outer mitochondrial membrane, the binding capacity of each VDAC isoform expressed in yeast mitochondria was assessed. When compared with the binding of hexokinase to mitochondria lacking VDAC, the results show that mitochondria expressing HVDAC1 are capable of specifically binding hexokinase, whereas mitochondria expressing HVDAC2 only bind hexokinase at background levels. The expression of each human cDNA has been assessed by Northern blot and polymerase chain reaction techniques. With one exception, each is expressed in all human cell lines and tissues examined.

The voltage-dependent anion channel (VDAC, also known as mitochondrial porin) of the outer mitochondrial membrane is a small (~30 kDa) abundant protein found in all eukaryotic kingdoms which forms a large (~3 nm) voltage-gated pore when incorporated into planar lipid bilayers (Colombini, 1988; Adams et al., 1991). Physiologically, VDAC is thought to function as the primary pathway for the movement of adenine nucleotides through the mitochondrial outer membrane, thus controlling the traffic of these essential compounds into and from the mitochondria as well as the entry of other substrates into a variety of metabolic pathways. VDAC has also been shown to be the binding site for hexokinase and glycerol kinase (Felgner et al., 1979; Linden et al., 1982; Fiek et al., 1982; Ostlund et al., 1983; Nakashima et al., 1986). Binding of these enzymes may allow them preferential access to mitochondrial ATP (Fiek et al., 1982; Ostlund et al., 1983; Seltzer and McCabe, 1984; Kaneko et al., 1985), although recently this view has been questioned (Kabir and Nelson, 1991). The association of hexokinase and glycerol kinase with mitochondria is dynamic, varying in different tissues, during development, and with respect to the metabolic state of the cell, suggesting that the association of these enzymes with the outer membrane constitutes a significant metabolic regulatory interaction (reviewed in Adams et al., 1991). Consistent with this notion, binding of these enzymes to VDAC may occur specifically at contact sites between the inner and outer mitochondrial membranes, thus linking cytoplasmic metabolism and ADP production as regulated by these enzymes with the regulation of mitochondrial respiration and oxidative phosphorylation in the mitochondrial matrix (Kottke et al., 1988; Brdiczka, 1990). In addition, cells in highly malignant tumors have an increased percentage of mitochondrial bound hexokinase when compared with normal cells (Nakashima et al., 1986), and it appears that VDAC is part of a complex forming the mitochondrial benzodiazepine receptor (McEnery et al., 1992).

Since VDAC is distributed throughout the outer membrane and hexokinase and glycerol kinase may be bound to mitochondria through VDAC only at contact sites, there has been speculation that there may be multiple forms of VDAC in mammals which differ in their localization within the outer mitochondrial membrane and in physiological function (Dorbani et al., 1987; Brdiczka, 1990). What appears to be a single VDAC protein in bovine (DePinto et al., 1987; De Pinto et al., 1991), rat (Roos et al., 1982; Colombini, 1983), and human

1 The abbreviations used are: VDAC, voltage-dependent anion channel; PCR, polymerase chain reaction; bp, base pair(s); MES, 1-morpholinoethanesulfonic acid.
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(Towbin et al., 1989; Thennes et al., 1989) tissue issues have been characterized, but VDAC genes have been cloned only from the fungi Saccharomyces cerevisiae (Mihara and Sato, 1985; Forte et al., 1987) and Neurospora crassa (Kleene et al., 1987). Comparison of the fungal proteins indicates that the amino acid sequence of VDAC is not highly conserved; the two fungal proteins share only 30% identity, although the two types of channels have very similar, if not identical, electrophysiological characteristics in planar lipid bilayers. Given this low level of sequence conservation, even within fungi, it is likely to be difficult to generate probes for the identification of mammalian VDAC genes from knowledge of the fungal sequences. In order to investigate the diversity of VDAC proteins in mammals and to begin to define the physiological significance of individual isoforms, should they exist, we have attempted to identify human VDAC cDNAs. In this report, we describe two cDNAs representing the transcripts of two different human VDAC genes. To confirm VDAC function, each protein has been expressed in yeast cells that lack the endogenous yeast VDAC gene. Human proteins isolated from yeast mitochondria formed channels with the expected characteristics of VDAC channels when introduced into planar phospholipid bilayers and can complement phenotypic defects associated with elimination of the endogenous yeast VDAC gene that has been expressed in yeast. Each isoform appears to differ in its ability to bind rat brain hexokinase. The expression of each transcript has also been assessed by Northern blot and PCR techniques. With one exception, both transcripts appear to be expressed in a wide variety of tissues.

MATERIALS AND METHODS

PCR Cloning of HVDAC1, Library Screening, and Identification of HVDAC2—HVDAC1 sequences were identified initially by PCR amplification of transcripts expressed in the human B-lymphocyte cell line WIL-2NS (provided by Miles Wilson, Oregon Health Sciences University) using nested primers based on the published amino acid sequence of a human lyphocyte VDAC protein. The primers used for the first round of amplification were GA(A/G)AA(A/G)TGGAA (Glnsg, sense) and NGC(G/A)TT(T/C)TTNCCNG(G/A)TC (Glnsg, antisense). Primers for the second round of amplification were GAGCTGTNG(T/C)ATGAATC (Gln sg, sense) and (G/A)TGCNGG(T/C)AT/G(A)AT(T/C)TGT (G/A)AT/T/C/TG (Glnsg, antisense). The first amplification reactions were performed for five cycles with a denaturing temperature of 96 °C for 30 s, annealing at 60 °C for 30 s, and elongation at 72 °C for 30 s. The products of this reaction were subjected to 45 cycles of amplification with a denaturing temperature of 96 °C for 30 s, annealing 37 °C for 60 s, and elongation at 72 °C for 30 s. Amplified fragments were subcloned into pBS(M13)-StrataClone and sequenced by standard dideoxy nucleotide chain termination methods using Sequenase (U. S. Biochemical Corp.). Fragments containing HVDAC1 sequences were used to screen a human pituitary lambda ggt 10 cDNA library (provided by D. Grandy, Volumn Institute) at high stringency. Hybridizations were carried out in 5 × SSC (1 × SSC = 0.15 M NaCl, 0.015 M sodium citrate), 50% formamide at 42 °C and filters washed in 0.1 × SSC at 65 °C. Restriction fragments from positive phage were subcloned and sequenced as described above.

HVDAC2 containing cDNAs were identified by low stringency hybridization of a human liver Agt11 cDNA library (provided by N. Kennaway, Oregon Health Sciences University). Duplicate filters were probed with restriction fragments containing the entire coding sequence of HVDAC1 either at high stringency or at low stringency (hybridization at 37 °C, wash at 30 °C, and at 37 °C, wash at 30 °C). Clones that hybridized at low stringency but not at high stringency were identified, subcloned, and sequenced as described above.

Expression of HVDAC1 and HVDAC2 in Yeast—For expression of human VDAC proteins in yeast, yeast VDAC sequences between the EcoRV and NsiI sites were replaced with corresponding human sequences. Expression in each case was driven by the yeast VDAC promoter, and each protein was expressed as a fusion protein in which the NH2 terminus of the human protein was replaced by the first 9 amino acids of yeast VDAC. For HVDAC1 oligonucleotide-directed mutagenesis was used to create an EcoRV site at codon 10 (C127) to A and T129 (C to G) (Fig. 1), converting this codon from Leu to Ile. The modified HVDAC1 gene was cut with EcoRV and NsiI and inserted into the yeast VDAC gene between the EcoRV and NsiI sites. The resulting fusion gene encodes amino acids 1-9 of yeast VDAC followed by amino acids 11-283 of HVDAC1. For HVDAC2, PCR was used to generate an EcoRV site at nucleotide positions 120-125. The resulting PCR product was digested with EcoRV, and the 850-bp fragment was inserted into the yeast VDAC gene between the EcoRV and NsiI sites. This construct encodes amino acids 1-9 of yeast VDAC followed by amino acids 229-294 of HVDAC2. Both constructs were inserted into single copy yeast plasmids and introduced into yeast lacking the endogenous yeast VDAC gene (Blachly-Dyson et al., 1990) by transformation. VDAC was prepared from yeast mitochondrial membranes and characterized electrophysiologically following insertion into planar lipid bilayers as described previously (Blachly-Dyson et al., 1990).

Hexokinase Binding—Hexokinase-1 for binding studies was purified from rat brain according to the method of Chou and Wilson (1972). For binding studies, mitochondria were prepared from yeast cells containing HVDAC1, HVDAC2, or lacking VDAC (Blachly-Dyson et al., 1990) and suspended in sucrose medium (0.25 M sucrose, 0.01 M Hepes, pH 7.4) to a protein concentration of 2 mg/ml. From this suspension, 0.1-ml aliquots were incubated for 30 min in an ice bath with increasing activity levels of hexokinase in the presence of 10 mM MgCl2 and 10 mM glucose. Following incubation, the mitochondria were separated from the supernatant by centrifugation in a Microfuge at 16,000 × g. The mitochondrial pellet was resuspended in 0.02 ml of phosphate buffer (10 mM, pH 7.2) supplemented with 10 mM glucose. Bound hexokinase activity was determined using radioactively labeled glucose (glucose-D-[3-3H], 16.8 Ci/mmol) by the method of Guggenheim et al. (1980) with the following modifications; 5 μl of the resuspended pellet was incubated for 10 min at 37 °C in triethanolamine buffer (50 mM, pH 7.2) containing 6 mM ATP, 8 mM MgCl2, and 2 mM glucose (ratio of unlabeled to radioactively labeled glucose = 70:1). The reaction was terminated by addition of 1 μl of 0.5M EDTA and subsequent centrifugation. A 20-μl aliquot of the supernatant was spotted on DE-81 filter paper, washed sequentially with water and 1 M glucose, and radioactivity counted in a liquid scintillation counter.

Human Expression—Expression of each HVDAC isoform was assessed by Northern blot analysis of total RNA and by PCR amplification of transcripts present in human cell lines and tissues. RNAs prepared from the indicated human cell lines and human liver, heart, hippocampus, pituitary, and thyroid were obtained from M Grompe, Department of Molecular and Medical Genetics, Oregon Health Science University, and R. Rehfuss, D. Grandy, and R. Cone, Vollum Institute. Full-length cDNAs were prepared from the indicated human cell lines and human liver, heart, and brain mitochondria by the yeast VDAC gene between the EcoRV and NsiI sites. This construct encodes amino acids 1-9 of yeast VDAC followed by amino acids 11-283 of HVDAC1 or 229-294 of HVDAC2. Expression of each transcript has also been assessed by Northern blot and PCR techniques. With one exception, both transcripts appear to be expressed in a wide variety of tissues.

RESULTS AND DISCUSSION

Identification and Characterization of HVDAC1 and HVDAC2—HVDAC1 encoding sequences were identified by use of two pairs of nested primers based on the published amino acid sequence of VDAC purified from human B-lymphocytes (Thennes et al., 1989; Kayser et al., 1989). First strand cDNA was prepared from transcripts expressed in a human B cell hybridoma and amplified in two stages using the nested primers to obtain a ~450-bp fragment. The se-
sequence of this fragment indicated that it contained an open reading frame encoding a polypeptide with a sequence identical to amino acids 83–230 of the published human VDAC protein sequence. This fragment was then used to screen a human pituitary cDNA library. One hybridizing clone (designated HVDA1) was identified and characterized. The sequence of the insert (Fig. 1) contains an open reading frame which encodes a polypeptide identical to the complete published protein sequence of the purified VDAC protein (Thinnes et al., 1989), with the addition of an amino-terminal methionine, the most 5' ATG codon in this cDNA. Since no in-frame stop codons exist in the putative 5' untranslated region, we cannot exclude the possibility that the nascent VDAC protein contains an amino-terminal extension that is subsequently cleaved, although yeast and Neurospora VDAC lack cleaved presequences, as do other mitochondrial outer membrane proteins. The open reading frame is followed by an SS4-3'-untranslated sequence containing a consensus polyadenylation signal (AAATAAA) at nucleotide 1788.

Genomic Southern blots probed with this cDNA at reduced stringency indicated that related VDAC sequences were likely to be present in humans (data not shown). To identify additional members of the family, a 750-bp HindIII fragment of HVDA1 containing most of the coding region was used to probe a human liver λ DNA library at both high and low stringency. Five clones that hybridized at low, but not at high, stringency were identified and characterized. The restriction map and nucleotide sequence of the ends of these clones indicated that they contained overlapping 5' sequences. Four of the clones were identical at the 5' end, each ending in a poly(A) sequence at position 1235, although no consensus (AAATAAA) site is present. The fifth clone contained an additional 168 nucleotides, including a consensus polyadenylation site at position 1387 followed by multiple A residues.
The identification of two classes of cDNA differing in the extent of 3'-untranslated sequences suggests the use of alternate polyadenylation sites. A combined cDNA sequence (hVDAC2) representing the longest 5' and 3' sequences is shown in Fig. 1. This sequence contains a single long open reading frame that extends from nucleotide 947 to nucleotide 974. Within this open reading frame, two ATGs are found near the 5' end, one at nucleotide 63 and a second at position 96. The ATG at position 63 shows a good (8/9) match with the yeast start codon consensus sequence (CCA/GCCATG(G)) consistent with the proposition that this is the translational start site, although the second ATG corresponds to the position of the proposed start codon in hVDAC1 and the start codons of the N. crassa and S. cerevisiae genes (Fig. 2).

Expression of hVDACs in Yeast and Physiological Characterization—One remarkable characteristic of VDAC channels is the extreme evolutionary conservation of basic channel properties. VDAC channels from yeast to mammals share similar (a) single channel conductances (roughly 4.5 nano-Siemens in 1 M KCl), (b) ion selectivity (about 2:1 preference for chloride over potassium), and (c) voltage sensitivity as measured by the steepness, $n$, of the voltage-dependent conductance changes (Benz, 1985; Colombini, 1989). In addition, all VDAC channels are symmetrical with respect to gating properties, i.e. VDAC channels are in their high conducting or "open state" at 0 mV and "close" to low conducting states at codon 11 and HVDAC2 at codon 22 (see "Materials and Methods"). These constructs were introduced into yeast cells lacking the endogenous yeast VDAC gene, mitochondria from the resulting transformants were isolated, and VDAC was purified from the mitochondrial membranes (Blachly-Dyson et al., 1990). Purified samples contained a single protein band lacking the endogenous yeast VDAC gene, mitochondria from the resulting transformants were isolated, and VDAC was purified from the mitochondrial membranes (Blachly-Dyson et al., 1990). Purified samples contained a single protein band of appropriate molecular weight as assessed by silver-stained SDS-polyacrylamide gels (data not shown). These proteins were then introduced into synthetic phospholipid bilayers and their electrophysiological properties tested (Blachly-Dyson et al., 1990).

As shown in Table I, the both human VDAC cDNAs when expressed in yeast form channels on reconstitution whose properties are characteristic of VDAC from other sources (i.e. single-channel conductance and selectivity). There were, however, qualitative differences between hVDAC1 and hVDAC2 channels. HVDAC1 produced typical VDAC channels with characteristic steepness of voltage dependence ($n$ values) of 2.2 ± 0.4 (positive potentials, three determinations) and 2.4 ± 0.2 (negative potentials, three determinations). The insertion of one channel voltage wave was applied. The dotted lines show extrapolations of the closed-channel current slope to zero current. Thus, the reversal potentials of both the open and closed channels are indicated by downward arrows. The steepness, $n$, of the voltage-dependent conductance changes (Benz, 1985; Colombini, 1989). In addition, all VDAC channels are symmetrical with respect to gating properties, i.e. VDAC channels are in their high conducting or "open state" at 0 mV and "close" to low conducting states at codon 11 and HVDAC2 at codon 22 (see "Materials and Methods"). These constructs were introduced into yeast cells lacking the endogenous yeast VDAC gene, mitochondria from the resulting transformants were isolated, and VDAC was purified from the mitochondrial membranes (Blachly-Dyson et al., 1990). Purified samples contained a single protein band lacking the endogenous yeast VDAC gene, mitochondria from the resulting transformants were isolated, and VDAC was purified from the mitochondrial membranes (Blachly-Dyson et al., 1990). Purified samples contained a single protein band of appropriate molecular weight as assessed by silver-stained SDS-polyacrylamide gels (data not shown). These proteins were then introduced into synthetic phospholipid bilayers and their electrophysiological properties tested (Blachly-Dyson et al., 1990).

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**Fig. 2.** Alignment of the amino acid sequences of HVDAC1 (H1) and HVDAC2 (H2) with those of yeast (S.C.) and *Neurospora* (N.C.) VDAC. Only identical residues have been boxed. Dashes indicate gaps of the amino acid sequence when compared with the other sequences.

**FIG. 3.** Properties of human VDAC channels expressed in yeast and inserted into planar phospholipid membranes. Both experiments illustrated were performed in the presence of a KCl gradient (1 M versus 0.1 M) solutions also contained 5 mM CaCl$_2$ and 1 or 5 mM Mes, pH 5.8. Experiments were performed under voltage-clamp conditions with VDAC inserting from the high salt side and voltages referring to the high salt side. On the left side of each recording, voltage steps were applied whose value (in mV) is indicated by the numbers. The insertion of one channel (lower figure) and two channels (upper figure) are indicated. On the right, a triangular voltage wave was applied. The dotted lines show extrapolations of the closed-channel current slope to zero current. Thus, the reversal potentials of both the open and closed channels are indicated by downward arrows.

**TABLE I**

|            | HVDAC1  | HVDAC2  | Yeast   |
|------------|---------|---------|---------|
| Single-channel conductance (nano-Siemens) in 1 M KCl | 4.1 ± 0.1 (6) | 4.0 ± 0.2 (6) | 4.2 ± 0.1 (3) |
| Single-channel conductance (nano-Siemens) in 0.1 M KCl | 1.87 ± 0.05 (4) | 1.76 ± 0.5 (3) | 1.80 ± 0.06 (4) |
| Reversal Potential in 1 M KCl at 0.1 M KCl vs 1 M KCl | 11.1 ± 0.6 (5) | 10.9 ± 0.2 (2) | 11.0 ± 0.2 (6) |
| Selectivity (P(Cl$^-$)/P(K$^+$)) | 1.8 | 1.8 | 1.8 |
and incubated at the indicated temperatures.

streaked on media containing et al., which the chromosomal VDAC gene was deleted formed strains on media containing glycerol. A yeast strain in events suggestive of subconductance states. With time, channels with properties characteristic of VDAC open channels appeared to lose their voltage dependence, whereas the voltage dependence of HVDAC2 channels persisted. Pre-

voltage dependence observed for HVDACZ channels may be an artifact produced by excision of native NH2-terminal domains and/or fusion of yeast NH2-terminal sequences.

Deletion of the VDAC gene from yeast leaves the cells viable, but results in temperature-sensitive growth on glycerol as the sole carbon source (two determinations; Fig. 3). In contrast, HVDAC2 channels often inserted as low conductance events suggestive of subconductance states. With time, channels with properties characteristic of VDAC open channels were observed with n values of 2.5–3.0 at both positive and negative potentials (two determinations; Fig. 3). These n values for HVDAC1 and HVDAC2 compare well with those obtained for yeast VDAC (2.5 ± 0.3, positive potentials, seven determinations; 2.4 ± 0.5 negative potentials, seven determinations). However, after 20 min to 1 h of testing, HVDAC2 channels appeared to lose their voltage dependence, whereas the voltage dependence of HVDAC1 channels persisted. Preliminary results suggest that expression of native HVDAC2 cDNAs (i.e. lacking NH2-terminal yeast sequences) in yeast results in the production of a very low level of channel activity with more stable voltage dependence. Thus, the instability of voltage dependence observed for HVDAC2 channels may be an artifact produced by excision of native NH2-terminal domains and/or fusion of yeast NH2-terminal sequences.

Deletion of the VDAC gene from yeast leaves the cells viable, but results in temperature-sensitive growth on glycerol-based media (Guo and Lauquin, 1986; Dihanich et al., 1987; Dihanich 1990).2 The physiological basis of the ability of strains lacking VDAC to conditionally utilize nonfermentable carbon sources is currently unknown. The exact phenotype resulting from elimination of the VDAC gene appears to be highly strain-dependent, and in some situations, a new channel has been reported to be present in the outer membrane that can functionally compensate for the lack of VDAC at low temperature (Dihanich et al., 1989; Michejda et al., 1989). In all cases, growth defects are corrected by reintroduction of a plasmid-based yeast VDAC gene. As shown in Fig. 4, introduction of the HVDC1 or HVDC2 constructs described above also complements the temperature-sensitive growth defect resulting from elimination of the endogenous VDAC gene. Thus, the human proteins we have identified not only produce channels with the characteristics observed for all VDAC channels, but the human genes can also functionally replace the yeast gene in yeast cells. We are confident then that these cDNAs encode human VDAC proteins.

One conserved characteristic of VDAC is its ability to provide a binding site for hexokinase to the outer mitochondrial membrane. To determine if the two human isoforms differ in their ability to bind this molecule, mitochondria prepared from yeast strains expressing HVDAC1, HVDAC2, or lacking any VDAC were compared. As shown in Fig. 5, the two isoforms differ in their ability to bind rat brain hexokinase; HVDAC1 binds hexokinase, whereas HVDAC2 only binds to the background levels observed in mitochondria lacking VDAC. This background level rat brain hexokinase binding is similar to the binding of mitochondria containing wild-type yeast VDAC (data not shown), presumably reflecting the species dependence of binding, since yeast VDAC has been demonstrated to bind yeast hexokinase (Forte et al., 1987). The observation that the two VDAC isoforms differ in their ability to bind hexokinase has clear implications for mechanisms of respiratory control involving the binding of this enzyme to the outer membrane, thereby allowing it preferential access (Adams et al., 1991) to mitochondrially generated ATP.

Expression of Genes Encoding HVDAC Isoforms—The potential for differential expression of HVDAC1 and HVDAC2 in a variety of human tissues and cell types was assessed by Northern blot analysis and specific PCR amplification. By Northern blot, both a 2-kilobase pair HVDAC1 transcript and a 1.3-kilobase pair HVDAC2 transcript are present at varying levels in three of the four cell lines tested and in RNA prepared from human thyroid (Fig. 6, A and B). Two

2 E. Blachly-Dyson and M. Forte, unpublished data.
HVDAC2 transcripts differing by 167 bp are expected due to alternate polyadenylation. If both are present, they are not resolved in this gel. HVDAC1 and HVDAC2 transcripts appear to be absent in the NB5 human neuroblastoma cell line. For PCR amplification, first strand cDNA was prepared from RNA isolated from a variety of tissues and cell lines and amplified using pairs of primers specific for the two isoforms (Fig. 7, A and B). HVDAC2 was detected as a 393-nucleotide amplification product in all tissues and cell types tested, including NB5 cells, where the level was likely to be too low to be detected by the less sensitive Northern blot technique. HVDAC1 was also detected by PCR in most tissues and cell types, with the exception of NB5 cells.

Sequence Comparisons—Alignment of the amino acid sequences encoded by the two human clones indicates that they are identical at 211/283 positions (75%), with no introduction of gaps and with an 11-amino acid amino-terminal extension in HVDAC2 relative to HVDAC1. The majority of the amino acid differences between HVDAC1 and HVDAC2 are conservative substitutions (e.g., Ser for Thr, Lys for Arg). The most dramatic difference between the two sequences is the presence of 2 acidic residues (Glu112 and Asp122) in HVDAC2 at the positions corresponding to two adjacent basic residues (Lys199 and Lys209) in HVDAC1. In addition, there are seven positions where one sequence contains a charged residue and the other an uncharged one (E36/C47, R93/Q104, H122/C133, and H126/Q137).
and human sequences is very similar, suggesting that they share similar overall structures. More likely then is the possibility that the exact position of one or more transmembrane strands, relative to the linear protein sequence, differs between yeast and human VDACs, such that regions that are aligned (in Fig. 2) with yeast loop domains form transmembrane strands in the human sequence. In the human channel, these “loop” regions contain excess positive charge and thus could balance the excess negative charge found in other transmembrane regions. For example, residues 104–122 of the human sequence (HVDAC1) contain 5 additional positively charged residues and a stronger overall transmembrane sided \( \beta \)-sheet pattern (Fig. 7) than the corresponding region of the yeast protein. It should be possible to test these alternatives by the construction of chimeric human/yeast VDAC channels and by site-directed mutagenesis of specific residues of human VDAC genes expressed in yeast.

Immunocytochemical studies using antibodies directed to the NH\(_2\)-terminal 19 residues of HVDAC1 have indicated that antibody cross-reacting molecules are present in the plasma membrane as well as the mitochondria (Thines et al., 1989; Babel et al., 1991). The identification of two human VDAC genes which encode proteins that are identical in 13 positions over this region raises the possibility that such antibodies recognize both isoforms. Thus, although both proteins can be directed to the mitochondria in yeast, in human cells one may also be preferentially expressed at the plasma membrane. Consistent with this notion, numerous reports have documented the presence of plasma membrane channels that have physiological characteristics similar to VDAC (Blatz and Magleby, 1983). Determination of the subcellular localization of each isoform awaits the development of reagents (i.e. antibodies) that specifically identify each isoform.

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