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Purification and Patch Clamp Analysis of a 40-pS Channel from Rat Liver Mitochondria†

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ABSTRACT: Patch clamp analysis of membranes reconstituted with a fraction isolated from detergent-solubilized mitochondrial membranes by affinity chromatography on immobilized quinine earlier indicated the presence of two classes of ion channels, of about 40- and 140-pS conductance in medium including 150 mM KCl. Now a 57-kDa constituent of the quinine-affinity column eluate has been identified as the 40-pS channel. Protein fractions derived from the quinine-affinity column eluate by preparative isoelectric focusing with a Rotofor cell have been reconstituted into phospholipid vesicle membranes by detergent dialysis, and vesicles have been enlarged for patch clamping by dehydration and rehydration. Voltage clamp analysis has been carried out on excised patches bathed symmetrically in buffered medium containing 150 mM KCl and 100 μM $CaCl_2$. Patches of membrane incorporating the 57-kDa protein exhibit 40-pS conductance transitions. The magnitude of conductance transitions is similar when Na^+ replaces K^+ in the bathing medium, indicating little selectivity of the 40-pS channel for K^+ relative to Na^+. Another fraction derived from the quinine-affinity column eluate is found to contain the larger channel, now estimated to have an average conductance of about 130 pS. Patches of control membrane prepared in the same way but without protein exhibit no channel activity.

The development of patch clamp techniques (Sakmann & Neher, 1983; Hille, 1984) has greatly facilitated the study of ion channels. Since the pioneering work of Sorgato et al. (1987), several laboratories have reported patch clamp studies of mitoplast membranes and of artificial membranes incorporating mitochondrial membrane fragments, indicating the presence of channels of conductance varying from 6 pS to 1.3

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nS in media containing 150 mM KCl or NaCl.

A voltage-dependent, slightly anion-selective channel of 107–108-pS conductance has been observed in patches of the inner mitochondrial membrane of rat liver and brown adipocyte mitochondria and in patches of reconstituted liposome membranes (Sorgato et al., 1987, 1989; Moran et al., 1990; Klitsch & Siemen, 1991; Inoue et al., 1991). The activity of this channel is reported to be enhanced by washing mitoplasts with EGTA (Kinnally et al., 1991). Purine nucleotides are found to be inhibitory (Klitsch & Siemen, 1991).

A giant 1.3-nS channel is found to be opened by millimolar levels of Ca^{2+} and blocked by cyclosporin A (Petronilli et al., 1989; Szabò & Zoratti, 1991). This channel may be responsible for permeability transitions induced in Ca^{2+}-loaded mitochondria by various reagents (Siliprandi et al., 1978; Beatrice et al., 1980; Broekemeier et al., 1989; Novgorodov et al., 1987, 1991; Rizzuto et al., 1987; Fournier et al., 1987; Carbonera & Azzone, 1988; Davidson & Halestrap, 1990; McGuinness et al., 1990). In other studies, conductance transitions ranging from 30 pS to 1–1.5 nS, interpreted as attributable to a common "multiconductance channel" (MCC), were found to decrease if free Ca^{2+} was kept at 10^{-7} M or lower during mitochondrial isolation or if mitoplast membrane patches were treated with drugs such as amiodarone, propranolol, and quinine (Kinnally et al., 1991; Antonenko et al., 1991). In possibly related studies, a water-soluble 60-kDa protein extracted from mitochondria in ethanol was reported to induce conductance increments in planar bilayers of various magnitudes from 24 to 175 pS (Mironova et al., 1981).

Conductance transitions of 30–50 pS have been recorded by several investigators (Kinnally et al., 1989; Klitsch & Siemen, 1991; Inoue et al., 1991; Wunder & Colombini, 1991). Such transitions have been attributed to a substate of the 108-pS channel (Klitsch & Siemen, 1991) or to the putative multiconductance channel (Antonenko et al., 1991). An ATP-sensitive K^+ channel of 10-pS conductance in the presence of 100 mM KCl has also been observed (Inoue et al., 1991).

A channel with multiple conductance levels at 220-pS increments, which may have a role in protein import, is blocked by a peptide identical in sequence to a mitochondrial targeting signal (Thieffry et al., 1988; Henry et al., 1989). This channel, which has been observed in a porin-deficient yeast (Fèvre et al., 1990), is found to be associated with the outer mitochondrial membrane (Chich et al., 1991). It has been suggested that most of the channel activity observed in patches of mitochondrial membranes may be attributable to activity of the outer membrane VDAC (porin channel), excepting 120-pS transitions exhibiting a voltage dependence distinct from that of VDAC (Wunder & Colombini, 1991). The conductance of VDAC differs between its open and closed states by about 450 pS in media containing 150 mM KCl (Wunder & Colombini, 1991).

A fraction isolated from detergent-solubilized mitochondrial membranes via affinity chromatography on immobilized quinine contains proteins estimated by SDS–PAGE to be 97, 77, 57, 53, and 31 kDa (Diwan et al., 1990). Quinine inhibits mitochondrial K^+ transport via apparent uniport and antiport mechanisms (Nakashima & Garlid, 1982; Jung et al., 1984; Diwan, 1986; Halestrap et al., 1986). Membrane vesicles reconstituted with the quinine-affinity column eluate were found to be permeable to K^+ and to the K^+ analogue Tl^+ (Diwan et al., 1988, 1990). On 2D gels, with isoelectric focusing as the first dimension, the major 53-kDa protein of the quinine-affinity column eluate was resolved into four bands of differing isoelectric points (Diwan et al., 1990). Sequencing of a 14 amino acid peptide derived from one of these bands indicated identity with part of the sequence of aldehyde dehydrogenase, an enzyme unlikely to have a role as an ion channel (Diwan et al., 1990). However, a 53–54-kDa protein, which tends to copurify with a 82-kDa K^+/H^+ antiporter, has been found to have K^+ uniport activity (Li et al., 1990; Hegazy et al., 1991).

Patch clamping of membranes reconstituted with the affinity-purified fraction, in the presence of buffered medium containing 150 mM KCl and 100 μM $CaCl_2$, indicated the presence of two classes of channels of about 40- and 140-pS conductance (Costa et al., 1991). The 140-pS channels were found to be voltage-gated, opening at positive voltages relative to the bath (negative pipet potentials). Such a finding implies asymmetric insertion of channels into vesicle membranes during reconstitution. This report describes the subfractionation of the quinine-affinity column eluate, with patch clamp analysis indicating assignment of the 40-pS channel to the 57-kDa protein.

Experimental Procedures

Protein Purification. As in earlier studies (Diwan et al., 1988, 1990), the affinity gel was prepared by reacting epoxy-activated Sepharose 6B (Pharmacia) with the OH group of quinine at alkaline pH. Submitochondrial particles prepared from 16 rat livers by standard procedures (Pedersen et al., 1978) and solubilized in 2% Triton X-100, 50 mM NaCl, 2 mM HEPES, and 10 mM EDTA (pH 7.5) were applied to two 4–5-mL affinity columns. Each column was washed with a similar solution in which NaCl was increased to 100 mM and the pH was 6.6. Protein was eluted with medium containing 1% sodium cholate, 400 mM KCl, and 2 mM Hepes (pH 6.4). Media included 0.5 μg/mL leupeptin and 0.7 μg/mL pepstatin. The eluate was concentrated to 2–3 mL by ultrafiltration in an Amicon stirred cell with YM-30 membrane and then diluted to 40 mL with medium containing 1% octyl glucoside, 5% glycerol, and 2% Bio-Lyte ampholytes (Bio-Rad, pH 5–8). Isoelectric focusing was carried out at 4 °C with a Bio-Rad Rotofor cell at 12-W constant power.

Selected Rotofor fractions (two to five adjacent fractions) were pooled and combined with octyl glucoside-solubilized asolectin [purified by the method of Kagawa and Racker (1971)] at a final concentration of 15 mg/mL asolectin and 200 mM octyl glucoside and dialyzed at 4 °C against 2 L of 25 mM KCl with 5 mM Tris–P_i, pH 7.5. During 64 h of dialysis, the medium was changed four times, with 8 g of Bio-Beads (SM-2, 20–50 mesh, Bio-Rad) added in a dialysis sac each time after the initial two changes of medium. Control vesicles were prepared the same way but with cholate elution medium or the Rotofor medium minus ampholytes substituted for Rotofor fractions. Each resulting vesicle suspension was passed through a separate 2-mL column of washed Bio-Beads (100–200 mesh) for removal of any residual detergent. Vesicles were then collected by ultracentrifugation at 50 000 rpm for 1 h 25 min in a Beckman SW60Ti rotor. In some experiments, to ensure obtaining vesicles with a protein concentration dilute enough for single channel analysis, a portion of each protein-containing vesicle suspension was mixed with twice the volume of control vesicle suspension prior to sedimentation.

Protein in the quinine-affinity column eluate was assayed using the bicinchoninic acid reagent from Pierce Chemical Co. Residual ampholytes in Rotofor fractions were found to interfere with colorimetric assays, even after treatments such as acetone precipitation in the presence of high salt or am-

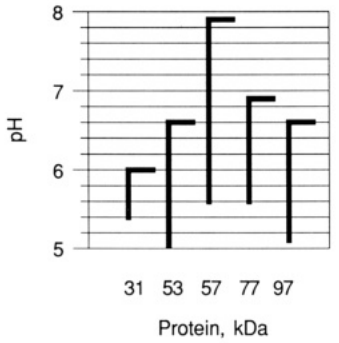

FIGURE 1: Distribution of proteins as a function of pH of Rotofor fractions. Protein bands at 31, 53, 57, 77, and 97 kDa were detected by their visibility on Coomassie blue-stained SDS gel lanes loaded with Rotofor fractions in one, six, six, four, and three out of six preparations, respectively. In three of these preparations the 31-kDa region of SDS gels was lost or obscured by ampholytes.

monium sulfate precipitation, expected to remove ampholytes (Garfin, 1990). Amounts of individual proteins in the affinity column eluate were estimated from the colorimetrically assayed total protein and the proportion of total Coomassie blue stain associated with each band in an SDS–polyacrylamide gel lane [system of Laemmli (1970)] loaded with the eluate. Gels were scanned with a Hoefer GS-300 densitometer, and peaks were integrated by the Hoefer GS-370 data system using a Macintosh computer. These estimates of relative protein contents are only approximate, because different proteins may bind the stain to differing degrees. By the same procedure, estimates of the amount of 57-kDa protein in Rotofor fractions were obtained by comparing integrated peak heights at 57 kDa in scans of adjacent gel lanes loaded with equal volumes of Rotofor and eluate samples.

Reconstitution and Patch Clamping. Vesicles prepared by detergent dialysis were enlarged by dehydration and rehydration, using procedures of Criado and Keller (1987), and collected in the medium to be used for patch clamping, usually 150 mM KCl, 5 mM HEPES, and 0.1 mM $CaCl_2$, pH 7.4. Aliquots were transferred to a clean culture dish. After 10 min vesicles not adhering to the dish were flushed off. A gigaohm seal was formed by bringing a patch pipet into contact with a vesicle and, if necessary, applying slight negative pressure to the pipet. Patches were excised by lifting the pipet away from the vesicle or by transient removal of the pipet from the bath. Pipets of 10–40-MΩ resistance prior to patching were prepared from borosilicate glass (1 mm with filament, World Precision Instruments) with a Sutter PC-84 pipet puller and used without further polishing or coating. An Axon Axopatch 200 amplifier was used. Filtering was at a bandwidth of 2 kHz. Current was recorded via a Medical Systems PCM-2 digitizer and stored on VHS tape. Data were analyzed on a BitWise 80386/SX computer, using Axon's pClamp software. Other apparatus included a Nikon TMS phase contrast microscope, Newport vibration isolation workstation, Narashige MO-303 micromanipulator, and Tektronix 2201 digital storage oscilloscope. Positive voltages reported refer to the interior of the pipet and correspond to negative bath potentials.

RESULTS

Protein Purification. The Rotofor procedure as carried out yields 20 fractions varying in pH from about 1.3 to 12.9, with most samples between pH 4 and pH 9. The distribution of protein bands, analyzed by SDS–PAGE, among fractions as a function of pH is summarized for six preparations in Figure 1. The minor 97-, 77-, and 31-kDa bands were not detected in Rotofor fractions in all preparations (see Figure 1 legend).

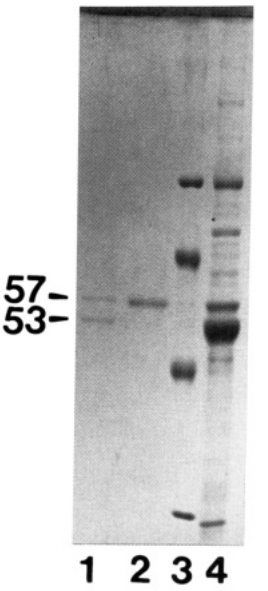

FIGURE 2: SDS–polyacrylamide gel stained with Coomassie blue: lane 1, pooled Rotofor fractions of pH 5.11–5.64; lane 2, pooled Rotofor fractions of pH 6.74–7.73; lane 3, MW standards (97.4, 66.2, 45, and 31 kDa); lane 4, quinine-affinity column eluate before focusing.

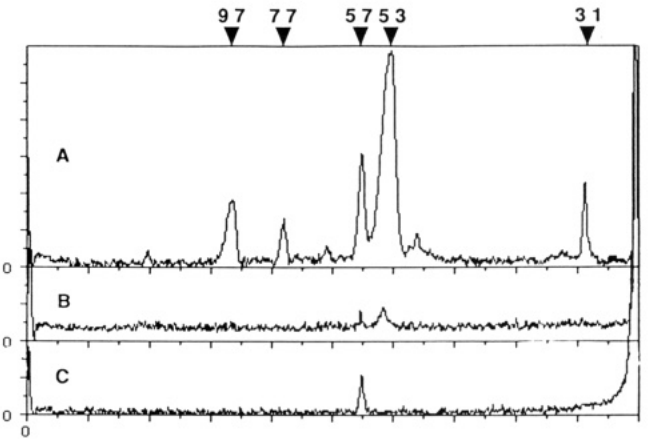

FIGURE 3: Scans of SDS gel lanes loaded with (A) quinine-affinity column eluate, (B) pooled Rotofor fractions of pH 5.11–5.64, and (C) pooled Rotofor fractions of pH 6.74–7.73. Same preparation but different gel from Figure 2.

Most of the proteins, when detected, were found to have overlapping distributions, but the 57-kDa protein consistently extended more into the alkaline pH range. Typically the 57-kDa protein spread over six to eight fractions of pH 5.6–7.9. Of the other proteins, only the 77-kDa band was found to extend above pH 6.6 to a pH 6.9 fraction in one of six preparations. When two to three low-pH fractions containing 53-kDa protein were discarded and the remaining fractions were pooled in the pH range from about 6.6 to 7.9, a sample which appeared as a single band on SDS gels at 57 kDa was usually obtained, as in the preparation depicted in Figures 2 and 3. Given the observed pH distributions, if any of the other proteins were present as a contaminant in those 57-kDa fractions which appeared to be pure, it was most likely the 77-kDa protein.

In some experiments, another pooled sample of lower pH fractions was obtained which appeared to contain only protein of 53 kDa. But usually one or more other bands were found in the 53-kDa fraction. In some preparations, the 57-kDa protein distributed so broadly that a fraction containing 53-kDa protein without substantial 57-kDa protein could not be obtained as in Figures 2 and 3. In contrast, on 2D poly-

Table I: Number of Membrane Patches Exhibiting Various Activities[a]

purified or predominant protein	total no. of patches	no obsd activity	transitions of about 40 pS	transitions of about 130 pS	app transitions of ≥200 pS	too noisy for analysis
57 kDa	49	19	22	5	5	3
53 kDa	30	16	3	9	2	2

[a] Combined data from five protein preparations. Rotofor fractions reconstituted were in all but one experiment within pH ranges 6.6–7.9 for 57-kDa protein and 5.1–5.8 for 53-kDa protein.

acrylamide gels [system of O'Farrell (1975)] loaded with urea-treated affinity column eluate, the 57-kDa protein consistently runs as a closely spaced cluster of bands to the alkaline side of the 53-kDa protein bands [unpublished data, and see Diwan et al. (1990)]. Perhaps in the Rotofor procedure as carried out individual 57-kDa protein molecules retain variable amounts of bound anionic cholate, resulting in the broad apparent range of isoelectric points.

Estimated by procedures summarized under Experimental Procedures, the yield of protein in the quinine-affinity column eluate derived from mitochondria of 16 rat livers was found to be usually 3–4 mg, with approximately 10% of this being 57-kDa protein. The yield of 57-kDa protein purified from this amount of eluate protein, after removal of about one-third of the Rotofor fractions for SDS–PAGE and after discarding fractions also containing 53-kDa protein, was found to be 21 and 60 μg in two preparations from which SDS–polyacrylamide gels suitable for precise scanning were obtained. This was the amount of 57-kDa protein combined in each experiment with 120 mg of phospholipid for membrane reconstitution. SDS–PAGE has confirmed incorporation of the 57-kDa protein into vesicle membranes (data not shown), but difficulty in recovering pellets after precipitation with acetone or chloroform/methanol has prevented an accurate quantitation of protein recovery in the vesicles. Residual phospholipid was also found to interfere with colorimetric assay of protein in vesicles, even after extraction with chloroform/methanol by the procedure of Barzilai et al. (1987).

SDS gels indicated a lower relative recovery of 53-kDa protein in the Rotofor fractions in most experiments. How the 53-kDa protein was lost or degraded during isoelectric focusing has not yet been explained. Precipitation was evidenced by turbidity only once among seven preparations, in a fraction containing 53-kDa protein.

Patch Clamp Analysis. Voltage-clamped patches of vesicle membranes reconstituted with the 57-kDa protein have been found to exhibit the channel of about 40-pS conductance (see Table I). Channel density was found to be approximately optimal for single channel analysis. Most patches appeared to contain no channel or one to two channels. A few patches were too noisy for analysis. Most of the patches of membrane incorporating 57-kDa protein which exhibited 130 pS and/or larger transitions were from a single protein/membrane preparation. A few such patches clearly contained the 130-pS channel. The transitions of 200 pS or larger tended to occur as occasional events or brief bursts of activity, representing a small fraction of the recordings. Patches of control membrane prepared without protein exhibited no channel activity (data not shown).

Figure 4 shows a representative recording from a patch of membrane reconstituted with the 57-kDa protein. Based mostly on observations of the channel in membranes prepared with the purified 57-kDa protein, but including a few measurements from membranes reconstituted with the whole quinine-affinity column eluate, the average conductance of this channel is found to be 44 ± 9 pS (mean of averages of multiple recordings from 21 active patches ± standard deviation).

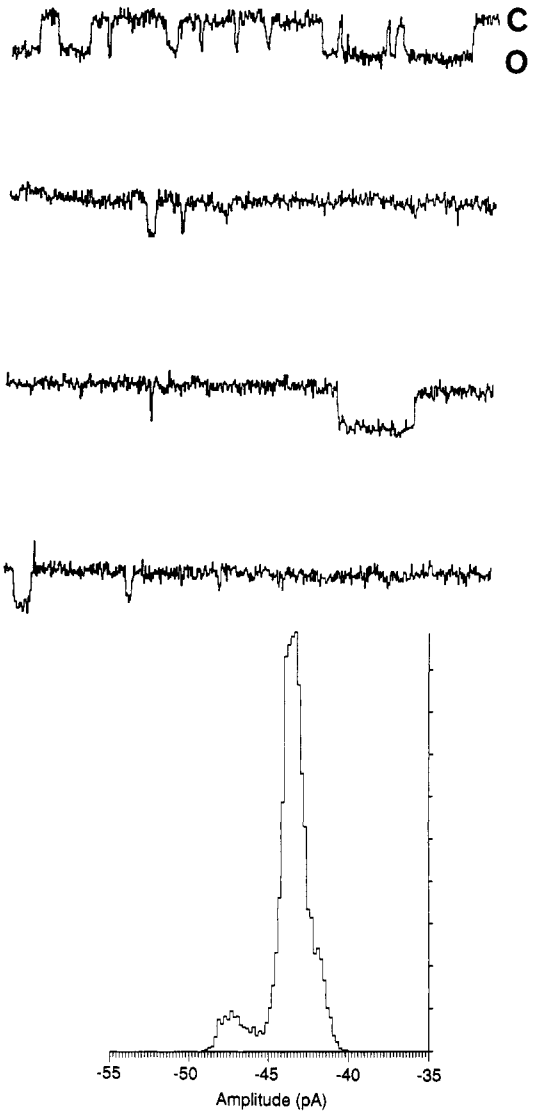

FIGURE 4: (A, top) Continuous recording of current vs time (102 ms/trace) at −80 mV from a patch of membrane reconstituted with the 57-kDa protein, showing transitions calculated to be 51 pS. Closed (C) and open (O) states are indicated. (B, bottom) All points amplitude histogram summarizing the recording partly shown in (A), displaying the relative occupancy of each pA current level.

Comparison of records obtained at different voltages over the range from −80 to +80 mV indicates no consistent variation in the magnitude of the conductance increment with voltage. Thus the channel in the open state appears to be ohmic. Although the open probability was found to vary from one patch to another, for a given patch the percent of time in the open or closed state showed no systematic variation with voltage. Thus the 40-pS channel does not appear to be voltage-gated.

As one test of selectivity, membranes reconstituted with the purified 57-kDa protein (or in one instance with quinine-affinity column eluate) were prepared in Na^+ medium, and excised patches were bathed on both sides with medium in-

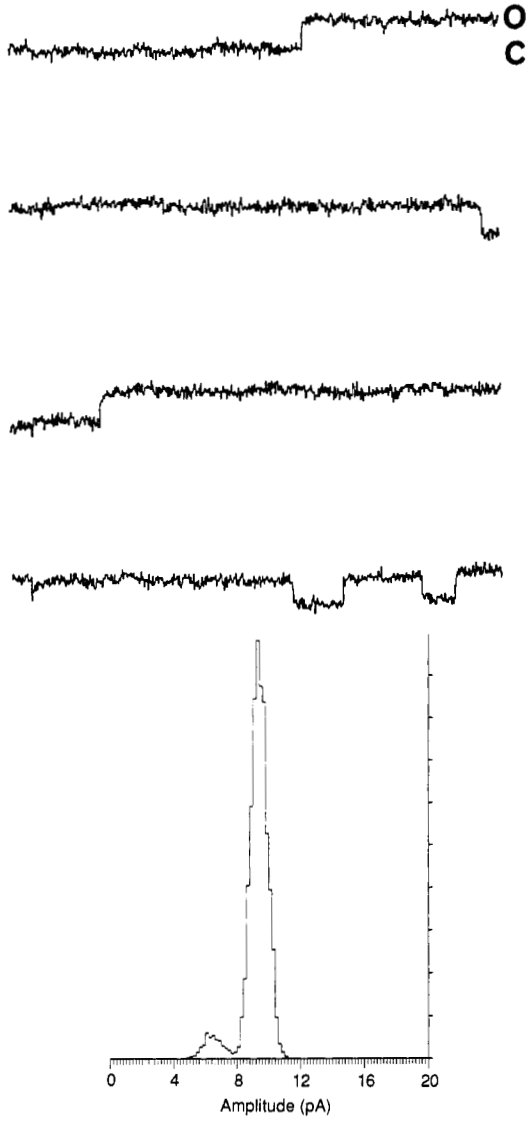

FIGURE 5: (A, top) Continuous recording of current vs time (102 ms/trace) at +60 mV from a patch of membrane reconstituted with 57-kDa protein, with Na^+ replacing K^+ in the bathing medium, showing transitions calculated to be 47 pS. Closed (C) and open (O) states are indicated. (B, bottom) All points amplitude histogram summarizing the recording partly shown in (A), displaying the relative occupancy of each pA current level.

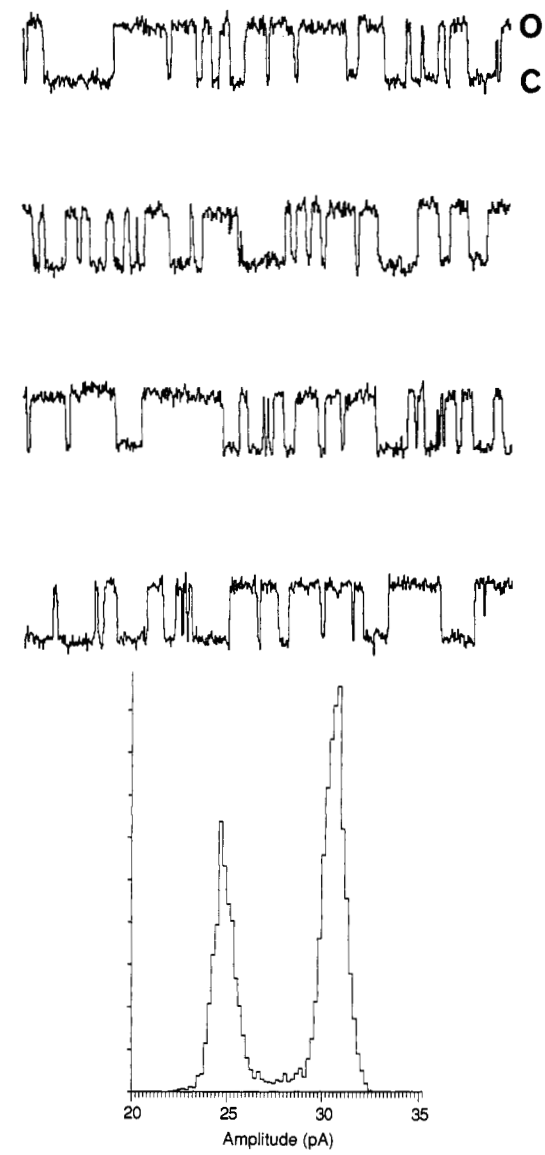

FIGURE 6: (A, top) Recording of current vs time (102 ms/trace) at +40 mV from a patch of membrane reconstituted with a fraction containing predominantly 53-kDa protein, showing transitions calculated to be 145 pS. Closed (C) and open (O) states are indicated. (B, bottom) All points amplitude histogram summarizing the recording partly shown in (A), displaying the relative occupancy of each pA current level.

cluding 150 mM Na^+ in place of K^+. The conductance in the presence of Na^+ was found to be 39 ± 9 pS (based on averages of values determined from multiple recordings from ten active patches), which is not very different from the conductance estimated in the presence of K^+. Such data suggest little selectivity for K^+ relative to Na^+. Figure 5 depicts a recording of activity of the reconstituted 57-kDa protein in Na^+ medium.

Considerable variation in kinetic parameters has been observed from patch to patch, but the 40-pS channel generally has been found to exhibit relatively long intervals between transitions. This is exemplified by mean open and closed times of 1.9 and 15 ms, respectively, for the recording of Figure 4 and 8.0 and 2.5 ms for the recording of Figure 5.

Patches of membranes reconstituted with pooled Rotofor fractions of pH 5.1–5.8 (extending to higher pH in one preparation), usually containing predominantly 53-kDa protein, exhibited activity of the larger of the two channels previously found to be associated with the quinine-affinity column eluate (see Table I). Activity of the 40-pS channel in membranes incorporating the lower pH fraction is likely to be underestimated, because flickering of the larger channel often results in relatively noisy recordings in which it is difficult to discern the 40-pS transitions. Pooled data from patches of membrane reconstituted either with the 53-kDa Rotofor fraction or with the quinine-affinity column eluate indicate an average conductance for the larger channel of 128 ± 19 pS (based on averages of multiple records from 24 active patches ± SD). A recording from a patch of membrane reconstituted with the fraction containing 53-kDa protein is shown in Figure 6. Although a majority of patches were inactive, suggesting low channel density, many active patches exhibited at least three current levels, interpretable as activity of two or more 130-pS channels. Such results imply aggregation or interaction of channels within the membrane.

No systematic variation of the magnitude of the approximately 130-pS conductance increments with voltage was observed over the range studied from −80 to +80 mV. For some patches, the open probability of the 130-pS channel was seen to vary with voltage as reported earlier (Costa et al., 1991), while with other patches an apparently opposite voltage de-

pendence was recorded. Some patches, many of which contained multiple 130-pS channels, exhibited no consistent voltage dependence. A possible explanation of these discrepancies would be variable transmembrane orientation of the channels. The basis for a more variable apparent orientation than in earlier studies is not clear. Recordings over a full range of voltages were not obtained from many individual patches, because of a tendency of the channels to become inactivated or exhibit silent periods, perhaps due to lateral diffusion into the membrane region sealed to the pipet.

Discussion

The data indicate that a mitochondrial protein estimated to be 57 kDa constitutes the 40-pS channel. The results do not rule out that some conductance transitions of similar magnitude might be attributable to the putative multiconductance channel (MCC) or a substate of the channel estimated to be 108 pS, as earlier proposed (Antonenko et al., 1991, Klitsch & Siemen, 1991). Patches of reconstituted vesicle membranes incorporating the 57-kDa protein occasionally have exhibited larger transitions that could be interpreted as activity of MCC or of the 130-pS channel (see Table I). But the fact that many patches of membrane reconstituted with the 57-kDa protein have exhibited only 40-pS transitions, even at the relatively high voltages reported to induce high-conductance states of MCC, supports the existence of a distinct 40-pS channel.

Assignment of the 130-pS channel remains uncertain because of the variable presence of contaminant proteins in the 53-kDa protein fraction exhibiting this activity. Evaluation of whether the 130-pS channel isolated by affinity to quinine corresponds to the voltage-gated 106–120-pS channel(s) detected in patches of mitochondrial membranes (Sorgato et al., 1987; Kinnally et al., 1989; Klitsch & Siemen, 1991; Inoue et al., 1991; Wunder & Colombini, 1991) must await completion of investigations of selectivity and pharmacology of the quinine-purified channel.

It would be premature to suggest possible physiological roles of the 40- and 130-pS channels, before channel selectivity and reagent sensitivity of gating have been thoroughly characterized. But one would expect the mitochondrial channels to be highly regulated, as are the channels of nerve plasma membranes, because the mitochondrial inner membrane is the site of essential metabolic functions dependent on a membrane potential (Mitchell, 1979).

Registry No. Na^+, 7440-23-5; K^+, 7440-09-7.

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Articles

Molecular Cloning of Genomic DNA and Chromosomal Assignment of the Gene for Human Aromatic L-Amino Acid Decarboxylase, the Enzyme for Catecholamine and Serotonin Biosynthesis†,‡

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ABSTRACT: Aromatic L-amino acid decarboxylase (AADC) catalyzes the decarboxylation of both L-3,4-dihydroxyphenylalanine and L-5-hydroxytryptophan to dopamine and serotonin, respectively, which are major mammalian neurotransmitters and hormones belonging to catecholamines and indoleamines. This report describes the organization of the human AADC gene. We proved that the gene of human AADC consists of 15 exons spanning more than 85 kilobases and exists as a single copy in the haploid genome. The boundaries between exon and intron followed the AG/GT rule. The sizes of exons and introns ranged from 20 to 400 bp and from 1.0 to 17.7 kb, respectively, while the sizes of four introns were not determined. Untranslated regions located in the 5′ region of mRNA were encoded by two exons, exons 1 and 2. The transcriptional starting point was determined around G at position −111 by primer extension and S1 mapping. There were no typical "TATA box" and "CAAT box" within 540 bp from the transcriptional starting point. The human AADC gene was mapped to chromosome band 7p12.1-p12.3 by fluorescence in situ hybridization. This is the first report on the genomic structure and chromosomal localization of the AADC gene in mammals.

Aromatic L-amino acid decarboxylase (AADC; EC 4.1.1.28) catalyzes the decarboxylation of both L-3,4-dihydroxyphenylalanine (L-DOPA) and L-5-hydroxytryptophan (L-5HTP) to produce dopamine (DA) and serotonin (5-HT), respectively (Lovenberg et al., 1962). DA and 5-HT are neurotransmitters in the central nervous system, and DA is further converted to norepinephrine and epinephrine, which are catecholamine neurotransmitters and adrenomedullary hormones. Thus AADC is an important enzyme for production of catecholamine and indoleamine, neurotransmitters, and hormones.

The distribution of AADC in peripheral tissues is different from that of other catecholamine-synthesizing enzymes. High AADC activity is found in the liver and kidney as well as in the adrenal medulla (Lovenberg et al., 1962; Rahman et al., 1981). Recent reports suggest that AADC in the kidney is responsible for intrarenal formation of DA, which regulates sodium excretion in proximal tubule cells (Hayashi et al., 1990). The physiological role of AADC in the liver, however, is still unknown.

Furthermore, APUD (amine precursor uptake and decarboxylation) cells (Pearse, 1969) also possess high AADC activity. Small cell lung carcinoma (SCLC) is an APUD cell and has a very high AADC activity, which serves to distinguish SCLC from other lung carcinomas (Baylin et al., 1980; Nagatsu et al., 1985).

Immunohistochemical studies showed the localization of AADC-like immunoreactivity in the brain. AADC-like immunoreactivity was found not only in catecholamine- and serotonin-containing neurons but also in a group of nonmonoaminergic neurons (Jaeger et al., 1983). These neurons are classified as D-type neurons and have no monoamine neurotransmitters. Natural substrates and the functional significance of AADC in these neurons are unknown. Tissue-specific expression of AADC activity in these cells is undoubtedly controlled by certain transcriptional factors.

Completely pure enzymes from pig kidney (Christenson et al., 1970) and human pheochromocytoma (Ichinose et al., 1985) decarboxylated both L-DOPA and L-5HTP. However, the arguments still exist whether or not the decarboxylation of L-DOPA and that of L-5HTP are mediated by the same enzyme, AADC. Some reports are against the identity of DOPA decarboxylase and L-5HTP decarboxylase from the aspects of the differential affinity for pyridoxal phosphate (a cofactor for the decarboxylation) (Siow & Dakshinamurti, 1985) and differences in kinetic parameters (Bouchard & Roberge, 1979).

Recent molecular biological research has revealed characteristics of this enzyme. We isolated and characterized a full-length cDNA clone encoding human AADC from a

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