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ARTICLE *in* BIOCHEMISTRY · DECEMBER 1992

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Oligomeric Properties of α-Dendrotoxin-Sensitive Potassium Ion Channels Purified from Bovine Brain†

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Received March 23, 1992; Revised Manuscript Received August 3, 1992

ABSTRACT: Neuronal acceptors for α-dendrotoxin (α-DTX) have recently been purified from mammalian brain and shown to consist of two classes of subunit, a larger (≈78 000 M_r) protein (α) whose N-terminal sequence is identical to that of a cloned, α-DTX-sensitive K^+ channel, and a novel M_r 39 000 (β) polypeptide of unknown function. However, little information is available regarding the *oligomeric* composition of these native molecules. By sedimentation analysis of α-DTX acceptors isolated from bovine cortex, two species have been identified. A minority of these oligomers contain only the larger protein, while the vast majority possess both subunits. Based on accurate determination of the molecular weights of these two forms it is proposed that α-DTX-sensitive K^+ channels exist as $\alpha_4\beta_4$ complexes because this combination gives the best fit to the experimental data.

K^+ channels are the most ancient and diverse group of ion channels in the nervous system (Hille, 1984). However, until recently the lack of specific and selective probes has restricted research into the structural features underlying this diversity. Fortunately, α-dendrotoxin (α-DTX)[1] and homologous peptide toxins from mamba venoms, which block a class of fast-activating, aminopyridine-sensitive K^+ channels in a variety of cells (Dolly et al., 1984; Halliwell et al., 1986; Benoit & Dubois, 1986; Stansfeld et al., 1987; Bräu et al., 1990), have allowed the purification of *putative* K^+ channels from rat (Rehm & Lazdunski, 1988) and bovine (Parcej & Dolly, 1989) brain. Analysis on SDS–PAGE of the isolated proteins from both species revealed the presence of two polypeptides, a large (α; 75 000–80 000 M_r) diffusely staining band which corresponds to the α-DTX-binding component identified by covalent cross-linking (Mehraban et al., 1984; Rehm et al., 1988; Scott et al., 1990) and a novel subunit (β) of M_r 37 000–39 000. Removal of the single sialic acid-containing N-linked glycan chain (Rehm, 1989; Scott et al., 1990) present on the larger subunit yields a core polypeptide with M_r 65 000 but does not reduce the broadness of this band on SDS–PAGE, implying the presence of isoforms; the β subunit is apparently not N-glycosylated. This heterogeneity of acceptors was initially unveiled by localization studies using radiolabeled α-DTX which binds specifically to both the myelin tracts and synaptic-rich regions of the brain, while a minor population of sites was identified using β-bungarotoxin (β-BuTX; a snake venom polypeptide from the Formosan banded krait) that binds avidly to a subtype of acceptor that resides predominantly in synaptic regions (Pelchen-Matthews & Dolly, 1989).

Concomitant with these biochemical investigations, a family of voltage-activated K^+ channels was cloned from mammalian central nervous system, using probes derived from the *Shaker* locus of *Drosophila* and the predicted sizes of these proteins approximate to that of the above-mentioned α subunit (Tempel et al., 1988; Stühmer et al., 1989; Swanson et al., 1990). Importantly, N-terminal microsequencing of the α subunit of purified α-DTX acceptors (Scott et al., 1990) identified an α subunit subtype almost identical to RCK 5, BK 2, and RBK 2 (Stühmer et al., 1989; McKinnon, 1989; Christie et al., 1990). Expression of each of the cloned genes in *Xenopus* oocytes yielded voltage-activated K^+ currents; some of these were sensitive to α-DTX (Stühmer et al., 1989), with RCK 5 being most susceptible. Unexpectedly, the expressed K^+ currents showed much slower inactivation kinetics than the transient, A-type currents produced by their *Drosophila* counterpart (Schwarz et al., 1988). The use of only single isoforms of the large subunit, wrong combinations (when tested) of the latter, or the absence of the smaller subunit described here and/or derived from the small RNA species reported by Rudy et al. (1988) may contribute to this discrepancy. It is, therefore, imperative that the subunit combinations and stoichiometries in the native K^+ channels be determined in order to interpret the extensive data on the expressed proteins in a more meaningful way. The present investigations on authentic K^+ channels purified from bovine brain showed them to be large oligomeric protein complexes with the bulk of these containing the α and β subunits in tight association. Accurate determination of the oligomeric size of this predominant form and that of a newly-discovered minor species, lacking the 39 000 M_r protein, allowed their subunit content to be deciphered.

EXPERIMENTAL PROCEDURES

Materials

Toxin I and α-DTX were purified from the venoms of *Dendrospis polylepis polylepis* and *D. angusticeps*, respectively, as described previously (Dolly, 1991); α-DTX was radioiodinated as detailed elsewhere (Black et al., 1986). Thesit was from Boehringer Mannheim; Triton X-114 and Tween 80 were purchased from Calbiochem. Enzymobeads were obtained from Bio-Rad and $Na^{125}I$ was from ICN. All other proteins and reagents were purchased from Sigma Chemical Co.

Methods

Acceptor Purification. α-DTX-binding proteins were purified from synaptic membranes of bovine cerebral cortex as detailed previously (Parcej & Dolly, 1989), with the

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[1] Abbreviations: PMSF, phenylmethanesulfonyl fluoride; DTT, dithiothreitol; α-DTX, α-dendrotoxin; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; M_r, relative molecular mass; R_s, Stokes' radius.

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following modifications: (i) the membranes were solubilized in extraction buffer containing Thesit (equivalent to Lubrol PX), and (ii) after loading of the extract onto the toxin I–Sepharose columns, subsequent steps employed buffer A [25 mM imidazole hydrochloride, pH 8.2, 0.1 M KCl, 1 mM EDTA, 0.2 mM benzamidine, 0.2 mM PMSF, and 0.05% (w/v) Tween 80] in order to maximize acceptor stability. Upon elution from the affinity resin, the acceptors were directly concentrated on a DEAE-Sepharose column (0.5 mL), equilibrated in buffer A, washed extensively to remove all DTT, and eluted in 0.3 M KCl in buffer A.

Radioiodination of α-DTX Acceptors. This was accomplished using Enzymobeads. After the beads had been resuspended in H_2O (50 μL) and allowed to swell for 1 h at 22 °C, 0.2 M potassium phosphate buffer, pH 7.2 (50 μL), 300 μCi of $Na^{125}I$ (3 μL of 100 mCi/mL), and 25–40 μL of purified acceptors (10–15 pmol) were added. The reaction was initiated by addition of 0.1 M α-D-glucose (25 μL); after 30–35 min of incubation at 22 °C, the reaction was halted and the labeled acceptors separated from free iodine by gel filtration on a Sephadex G-50 (fine) column equilibrated in buffer A. The sample was rechromatographed on a small DEAE-Sepharose column (as detailed earlier), taking precautions to eliminate all the radioactive detergent by extensive washing before the elution step. The samples were stored at 4 °C; prolonged storage of the labeled acceptors resulted in aggregation.

Hydrodynamic Studies. Linear sucrose gradients were prepared as detailed elsewhere (Black et al., 1988) with the following improvements. The sucrose solutions were prepared in buffer A containing 0.2% (w/v) Tween 80. Active acceptors (10 pmol/mL) were concentrated ≈20-fold in a microconcentrator (Centricon 100 from Amicon). Aliquots (200 μL) of native and radioiodinated acceptor (10 pmol and 100 000 cpm, respectively) were carefully loaded, together with standard protein markers, onto H_2O and ^2H_2O gradients and centrifuged at 190 000*g* (39 000 rpm) for 14 h in a Beckman SW40 Ti rotor. Fractions (250 μL) were assayed for ^{125}I-α-DTX binding or radioactivity was monitored by γ-counting. Gel filtration of the two acceptor samples (as above) was performed on a Sephacryl S400 HR column (1 × 42 cm) equilibrated in buffer A, together with standard protein markers. Analysis of data from density gradients and gel filtration was by the methods of Meunier et al. (1972) [see Black et al. (1988)] and Ackers (1967), respectively.

Phase Separation of Bovine Brain K^+ Channels. The purified K^+ channels were subjected to phase separation in Triton X-114 solution by the method of Bordier (1981). The protein preparation (2 pmol of ^{125}I-α-DTX binding; 25 μL in buffer A) was added to 245 μL of buffer P (buffer A lacking detergent), made 0.5% (w/v) with Triton X-114, and incubated on ice for 5 min. The mixture was layered onto a 0.3-mL cushion of 6% (w/v) sucrose in buffer P plus 0.06% (w/v) Triton X-114, warmed to 30 °C and centrifuged for 3 min at 300*g*. The detergent-poor phase (upper layer) was collected and subjected to a second separation using the same sucrose cushion. The detergent-poor phase was rinsed as described by Bordier (1981) and both phases were diluted to 1 mL with buffer P containing 0.5% (w/v) Tween 80. Aliquots were assayed for ^{125}I-α-DTX binding or precipitated (Wessel & Flugge, 1984) for analysis by SDS–PAGE.

Other Methods. SDS–PAGE was performed as described previously (Scott et al., 1990) and gels were calibrated by the method of Peterson et al. (1986). ^{125}I-α-DTX binding was determined as detailed elsewhere (Parcej & Dolly, 1989).

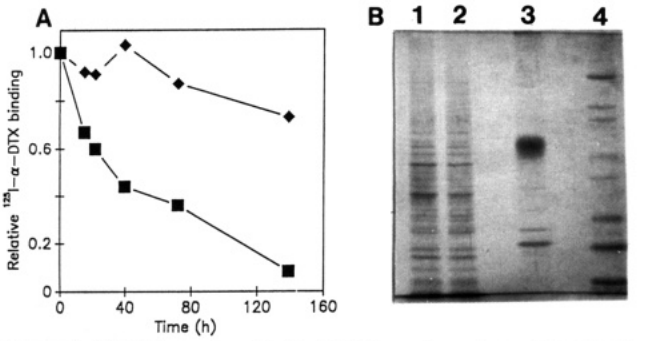

FIGURE 1: Stability and purity of α-DTX acceptors chromatographed in Tween 80. α-DTX acceptors were purified on a 2-mL toxin I–Sepharose column as detailed in Experimental Procedures. (A) Aliquots were assayed for saturable ^{125}I-α-DTX binding after storage at 4 °C for the times shown; samples were prepared using either 0.05% (w/v) Thesit (■) or 0.05% (w/v) Tween 80 (◆) in the column buffers. (B) SDS–PAGE of samples at various stages of the purification performed using Tween 80. Lane 1, crude extract; lane 2, breakthrough of toxin I–Sepharose column; lane 3, DTT eluate of the latter column; lane 4, standard proteins (thyroglobulin, 330 000; α_2 macroglobulin, 161 000; β-galactosidase, 116 000; phosphorylase *b*, 97 000; bovine serum albumin, 66 000; catalase, 57 000; ovalbumin 43 000; glyceraldehyde-3-phosphate dehydrogenase, 36 000; carbonic anhydrase, 29 000).

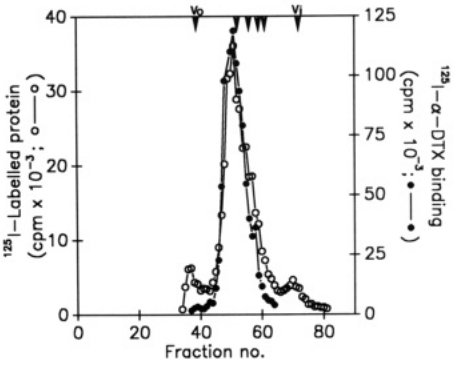

FIGURE 2: Gel filtration of purified α-DTX acceptors. Unlabeled and radioiodinated α-DTX acceptors, purified on a toxin I–Sepharose column, were chromatographed on a Sephacryl S400 HR column (1 × 42 cm) at a flow rate of 15 mL/h. Fractions (0.5 mL) were collected and assessed for radioactivity or saturable ^{125}I-α-DTX binding. The elution positions of standards (Stokes' radii nanometers) are shown by arrows: thyroglobulin, 8.6; apoferritin, 6.4; catalase, 5.2; yeast alcohol dehydrogenase, 4.6. V_0 and V_i indicate void and inclusion volumes.

RESULTS

Enhanced Stability of Purified K^+ Channels. By judicious selection of detergent (Lubrol PX), α-DTX acceptors may be solubilized in a form that is stable in the extract for several days (Parcej & Dolly, 1989). However, after affinity chromatography, acceptors isolated in Thesit (or Lubrol PX) lost ≈40% of their ^{125}I-α-DTX binding activity upon overnight storage at 4 °C (Figure 1A); addition of 10% (w/v) glycerol or replacement of Thesit by the related polyoxyethylene detergents, Genapol C-100 and X-150, did not reduce this loss. In contrast, use of 0.05% (w/v) Tween 80 as the detergent in the column wash and elution buffers maintained activity for several days after purification (Figure 1A). The acceptor isolated in this medium gave a subunit composition on SDS–PAGE (Figure 1B) identical to that obtained previously (Parcej & Dolly, 1989; Scott et al., 1990). Similarly, the preparation was judged to be oligomerically pure by gel filtration (Figure 2); the elution of active acceptors coincided with total protein as determined by radioiodination (see later). Tween 80 was, therefore, used for all further experiments.

Behavior of Purified α-DTX Acceptors in Triton X-114. Phase partitioning in Triton X-114 is a useful technique for

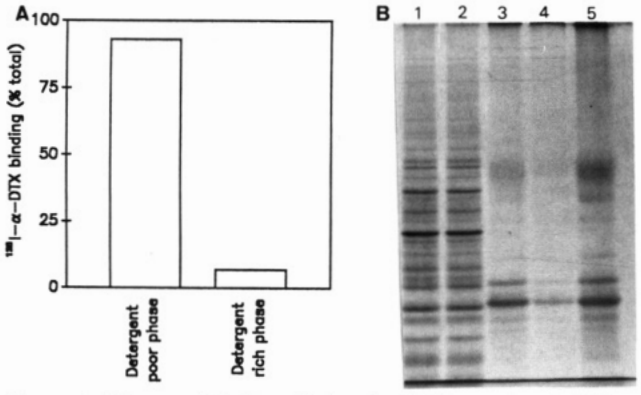

FIGURE 3: Phase partitioning of isolated α-DTX acceptors in Triton X-114. The purified protein was diluted with 0.5% (w/v) Triton X-114 and phase separation was performed as described earlier (see Methods). Distribution of the acceptors between the detergent-rich and -poor phases was determined from measurement of ^{125}I-α-DTX binding (A) or by analysis on SDS–PAGE (B). Tracks 1, membrane detergent extract; 2 and 3, breakthrough and eluate of the affinity column; 4 and 5, detergent-rich and detergent-poor phases, respectively, of the purified acceptor after being subjected to phase separation.

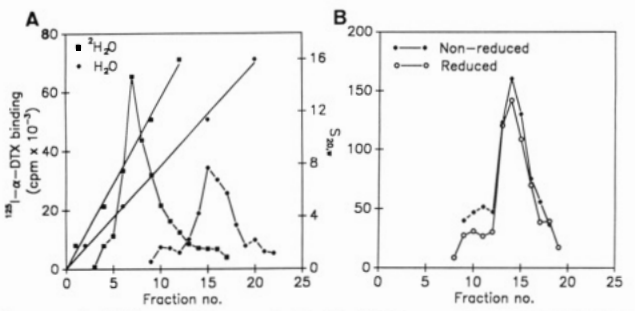

FIGURE 4: Sedimentation analysis of α-DTX acceptors. (A) Purified α-DTX acceptors were sedimented through linear sucrose gradients prepared in ^1H_2O (●) or ^2H_2O (■) and detected by ^{125}I-α-DTX binding. Gradients were calibrated using standard protein markers (cytochrome *c*, 1.8 S; equine liver alcohol dehydrogenase, 4.8 S, catalase, 11.3 S; and β-galactosidase, 16 S) and the resulting data were analyzed as detailed by Meunier et al. (1972). (B) The preparation was reduced by incubation for 30 min with 100 mM DTT and centrifuged through a linear sucrose gradient in H_2O containing 10 mM DTT (○). Fractions were assayed for ^{125}I-α-DTX binding after rapid gel filtration on G-75 (Parcej & Dolly, 1989) to remove DTT. A nonreduced sample was analyzed on a parallel gradient deovid of DTT (●).

Table I: Physical Properties of Two Forms of α-DTX Acceptors[a]

	large	small
apparent $S_{20,w}$ (^1H_2O)	11.2 ± 0.81	10.1
apparent $S_{20,w}$ (^2H_2O)	9.9 ± 0.68	10.5
partial specific volume (mL/g)	0.78 ± 0.004	0.72
$S_{20,w}$ (S)	16.1 ± 0.2	9.6
Stokes' radius (nm)	8.6	7.8
molecular weight of acceptors/detergent complex	680 000 ± 35 000	303 000
weight fraction (g of detergent bound/g of protein)	0.53–0.61	0.79–0.08
molecular weight of acceptors	370 000–420 000	240 000–265 000

[a] Values [determined by sucrose density gradient centrifugation and gel filtration (see Experimental Procedures)] are expressed ± SD or as a mean of duplicate experiments (error less than 5%). Acceptors were monitored by ^{125}I-α-DTX binding activity (large form) or γ-counting (small form). The partial specific volume of Tween 80 was taken to be 0.89 mL/g, and that of the acceptors, 0.70–0.73 mL/g [Na^+ channel and nicotinic acetylcholine receptor, respectively, as calculated from their amino acid compositions (Miller et al., 1983; Vandlen et al., 1979)]. In order to correct for attached carbohydrate, the degree of glycosylation was assumed to be that for the Na^+ channel [30% (w/v) hexose; v = 0.61 mL/g].

separating integral membrane proteins from those which are hydrophilic (Bordier, 1981). When subjected to this protocol, only ≈20% of the ^{125}I-α-DTX binding activity in the purified preparation was recovered, consistent with the loss of activity upon purification in this detergent (not shown). However, the remaining activity clearly partitioned into the detergent-poor phase, with only ≈5% found in the detergent-rich phase (Figure 3A). SDS–PAGE analysis of the protein in each phase (Figure 3B) showed the activity to be representative of the intact acceptor as both subunits appeared in the aqueous layer, the subunit pattern being qualitatively similar to the starting material. Although a residual amount of Tween 80 [≤0.01% (w/v)] was present in the initial protein preparation, it would not be expected to deter partitioning in Triton X-114 (Bordier, 1981). This has been substantiated in the case of bacteriorhodopsin; when the protein was suspended in Tween 80, diluted in Triton X-114, and treated as above, it was found exclusively in the detergent-rich phase. Thus, α-DTX acceptors appear to behave as hydrophilic proteins with both subunits comigrating, despite the six membrane-spanning helical regions presumed to be present in the α subunit, together with the recently documented hairpin loop (Yool & Schwarz, 1991; Yellen et al., 1991; Hartmann et al., 1991).

Hydrodynamic Properties of α-DTX Acceptors. Assaying fractions from the sucrose gradients for ^{125}I-α-DTX binding identified a single peak in ^2H_2O; however, in H_2O a minor peak (Figure 4) preceded the major one. The recovery of binding activity in ^2H_2O was greater than in H_2O, as was previously reported for the solubilized acceptor (Black et al., 1988). The apparent sedimentation coefficient of the major peak was determined by comparison with standard proteins as 11.2 and 9.9 S in H_2O and ^2H_2O, respectively. A plot of the distance travelled by the standard proteins against $S_{20,w}(1 - \bar{v}\rho_{1/2})$ gave a straight line with slope k, for both solvents, which was then used to determine the $S_{20,w}$ value of the major species as 16.1 S (Table I). The Stokes' radius (R_s) value for the major species was determined by gel filtration (Figure 2) to be 8.6 nm, in agreement with the value reported for the solubilized acceptors (Black et al., 1988). The molecular weight of the acceptor/detergent complex was calculated to be 680 000, and upon correction for bound detergent, the size of the large form of the acceptors was found to be 370 000–420 000. To exclude the possibility that the large form results from disulfide linkage of two of the smaller species (cf. the acetylcholine receptor from *Torpedo* membranes), centrifugation was repeated under reducing conditions (Figure 4B). Equivalent ^{125}I-α-DTX binding activity, measured in the two peaks under both conditions, established that the acceptors present in the larger peak are not disulfide-linked dimers of the minor form. Rather, two distinct oligomeric types are present.

To facilitate further investigation of the acceptors, a method for radioiodination of the protein was optimized. Relatively high incorporation of ^{125}I-α-DTX into the protein (≈1 × 10^7 dpm/pmol) was obtained under conditions designed to minimize oxidative damage. Analysis of the radiolabeled acceptors on SDS–PAGE (Figure 5B) identified clearly a 58 000 M_r protein, in addition to the subunits observed by silver staining (Figure 1B), presumably due to its relatively high level of incorporation of ^{125}I. Using type-specific anti-RCK 2 antibodies, this band has been identified as the bovine equivalent of RCK 2 (Grüpe et al., 1990), an α-DTX-sensitive member of the mammalian *Shaker* K^+ channel family (data not shown). In fact, use of other type-specific antibodies against fusion proteins corresponding to selected sequences from RCK 1, 4, and 5 established that all of these bovine equivalent α subunit subtypes are present, to different extents,

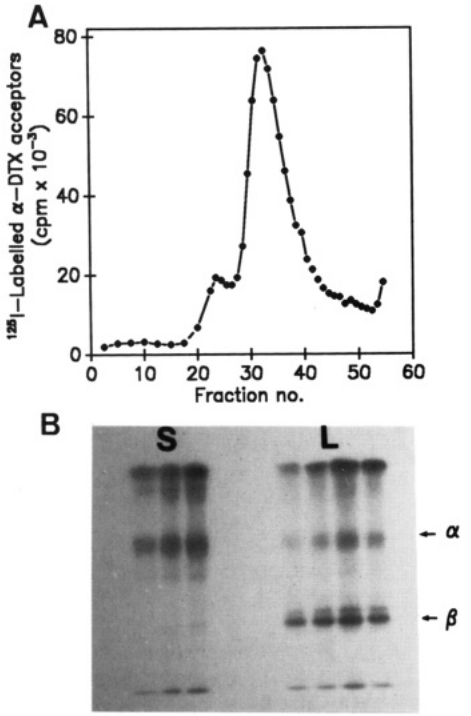

FIGURE 5: Separation of two forms of α-DTX acceptor by sucrose density gradient centrifugation. A radiolabeled sample of the purified acceptor was centrifuged through a linear sucrose gradient (5–20%); each fraction (0.25 mL) was assayed for radioactivity by γ-counting (A). The two peaks containing the small (S, three fractions) and large (L, four fractions) were then analyzed for subunit content by SDS–PAGE (B), followed by autoradiography. Arrows indicate the mobility of the α and β subunits.

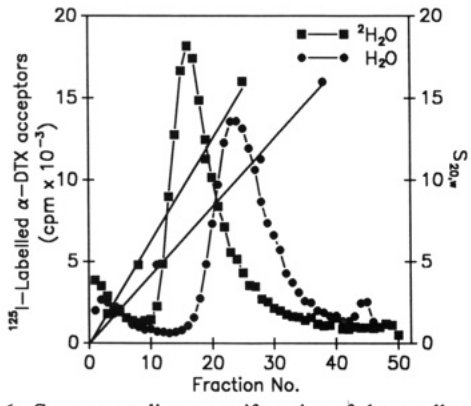

FIGURE 6: Sucrose gradient centrifugation of the smaller acceptor form. The lower-sized radiolabeled peaks from several sucrose gradients (Figure 5) were pooled and recentrifuged on linear sucrose gradients in $^{1}H_2O$ (●) or $^{2}H_2O$ (■), together with standard proteins (as in Figure 4A). Acceptors were monitored by γ-counting of the entire fraction (0.25 mL).

in the purified K+ channel preparations (Dolly et al., 1992). The labeled acceptors were subjected to sucrose gradient centrifugation as before, yielding essentially the same pattern as for the active acceptor (Figure 5A). SDS–PAGE analysis followed by autoradiography (Figure 5B) showed that the major species contained all the subunits present in the purified preparation; however, the minor species lacked the β subunit. Clearly, this represents a simplified form of the K+ channel complex.

To determine the physical properties of this small acceptor form, material from several sucrose gradients (in H_2O to ensure adequate separation) was collected, recentrifuged on $^{2}H_2O$ and H_2O gradients (Figure 6), and gel-filtered as before. A molecular weight of 240 000–265 000 was obtained for the protein moiety (Table I). As this size can be accounted for by four of the α subunits, the smaller form is likely to be a tetramer. Furthermore, the difference in mass between the large and small forms is consistent with the additional presence of four of the β subunits. Attempts to reconcile the determined sizes of the oligomers with various other combinations of α β subunit (e.g., $\alpha_4\beta_3$) failed to yield as good a fit to the observed values. Therefore, the most likely stoichiometry of the predominant form of purified K+ channels is 4:4 α/β subunits.

DISCUSSION

Replacement of Lubrol PX with Tween 80 in the purification procedure significantly extends the lifetime of isolated α-DTX acceptors. This difference in stability could reflect the extremely low critical micelle concentration of Tween 80 (12 μM; Tanford & Reynolds, 1976) or may be a function of its different structure. Since α-DTX acceptors are integral membrane strucures (Parcej & Dolly, 1989), it might be expected that they would partition into the detergent-rich phase during Triton X-114 phase transition. However, ≈95% are found in the aqueous, hydrophilic phase. A similar distribution was observed for the glycine receptor from spinal cord (Schmitt et al., 1987) and the acetylcholine receptor from *Torpedo* electroplax (Maher & Singer, 1985). The latter authors suggest that this counterintuitive behavior may be a general feature of ion-translocating proteins and could reflect discontinous, membrane-spanning surfaces preventing insertion into the relatively rigid Triton X-114 micelles, a prerequisite for partitioning into the detergent-rich phase. As both subunits comigrate in this system (cf. Rehm, 1991), it is not possible to assign their relative disposition in the membrane on the basis of these results.

In accord with studies on crude solubilized α-DTX acceptors (Black et al., 1988), a molecular mass of 370 000–420 000 Da was obtained for the purified material. However, in addition, a much smaller form, lacking the M_r 39 000 subunit, was identified and its molecular mass was determined to be 240 000–265 000 Da. Although the functional significance of this protein is not clear, it has allowed deduction of the oligomeric structure ($\alpha_4\beta_4$) of the native K+ channel. Importantly, the vast majority of these channels contain both large and small subunits and are likely to represent the dominant species in the membrane. It should be noted that the tetrameric structure proposed previously (Stühmer et al., 1989) for the functional K+ channels, observed upon expression of the α subunit alone, was based on the fact that the much larger α subunits of voltage-activated Na^+ and Ca^{2+} channels each possess four repeat domains; also, this model neglected the inclusion of smaller proteins known to be associated with these channels. Importantly, an experimentally-derived oligomeric structure is reported here, for the first time, showing the *authentic* K+ channels isolated from neuronal membranes are normally composed of four copies of both the α and β subunits. This is a very significant finding for future understanding of structure–activity relationships of this family of K+ channels and may also prove to be relevant to other cation channels. Notably, our proposed model is consistent with the tetrameric structure extrapolated for K+ channels formed by coexpression in oocytes of wild-type and mutant (insensitive to charybdotoxin) forms of the *Shaker* gene, using defined ratios of each (MacKinnon, 1991). However, the results presented herein extend this structural model to include the β subunit found in the predominant form of the protein from mammalian brain but not reported, as yet, for the *Shaker* K+ channels.

The functional importance of the β subunit in the bovine K+ channel is highlighted by a recent series of elegant studies showing the diversity of roles served by subsidiary subunits

(β, γ, α_2/δ) in voltage-activated Ca^{2+} channels, expressed in oocytes (Mori et al., 1991; Singer et al., 1991; Gutierrez et al., 1991). These include altering the level of expression as well as changing the channel's biophysical properties (e.g., activation or inactivation kinetics, peak conductance). Interestingly, these effects were particularly dramatic when certain combinations of the auxiliary proteins were used with a particular α_1 subunit; for example, a distinct pattern of changes was produced when the smaller subunits from skeletal muscle were expressed with α_1 from cardiac tissue. It is becoming apparent that the Ca^{2+} channels in all tissues examined possess equivalent counterparts to the small subunits from muscle. Thus, in view of the evidence presented in our study for a heterooligomeric structure for K^+ channels, this molecular architecture may typify all such voltage-activated cation channel proteins.

It is clear from these considerations that cloning of the β subunit and coexpression with the α subunit variants will be necessary to determine the variety of combinations of these subunits that could occur in voltage-dependent K^+ channels. The conclusive evidence for the existence of several isoforms of the α subunit, both from the multiplicity of genes cloned from brain and from the biochemical investigations (see the introduction), implies quaternary structure variation in α-DTX acceptors. Clearly, each α form is capable of forming functional K^+ channels either as homomultimers or as heterooligomers, with or without the β subunit. Perhaps, this highly desirable approach might show that the presence of the β subunit in an oligomeric complex of particular α subtypes is a prerequisite for obtaining a K^+ channel subtype to which β-bungarotoxin can bind, thereby explaining why the latter has not yet been observed with expression studies even though such proteins are readily detectable in brain. Such a postulated mechanism for creating an array of oligomers could underlie the wide diversity of the voltage-dependent K^+ channels found in neuronal membranes by electrophysiological analysis and using α-DTX or homologues as probes.

Certainly, much further experimentation is needed to validate this proposal and to establish how the differential expression of the multiple α subunits is controlled in different cell types and their assembly with β subunits (a process which the latter might control). Such information is fundamental to establishing the mechanisms enabling membranes to produce their characteristic K^+ channel variants. Similarly, it will be intriguing to investigate the means by which a particular channel form is directed to a given cellular location (somatic, dendritic, axonal, or synaptic membrane). In this context, the likelihood of the β subunit residing on the inner leaflet of the membrane, and being instrumental in interaction with the cytoskeleton, is an attractive possibility, as already proposed for the β subunit of L-type Ca^{2+} channels (Catterall, 1991).

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