Electrophoretic Analysis of Basal Body (Centriole) Proteins†

Richard G. W. Anderson* and Anna K. Floyd

ABSTRACT: Purified basal bodies isolated from the chicken oviduct were analyzed by using several different electrophoretic techniques. For comparison, oviduct cilia proteins were also analyzed. Prominent among the basal body proteins were the tubulin subunits (representing ~20% of the protein) and a low molecular weight protein (~17 400 daltons). In addition, major bands were present with molecular weights of ~180 000 and ~90 000. Electrophoretically purified basal body tubulin subunits had isoelectric points of 5.45 (α subunit) and 5.1 (β subunit). In addition, these isoelectric focus gels contained at least four other proteins that had higher isoelectric points, which indicates that tubulin purified by one-dimensional electrophoresis contains other proteins. On the basis of several different electrophoretic techniques, it was found that basal body tubulin differed from cilia tubulin even though they both had similar isoelectric focusing points. Whereas basal bodies did not contain any proteins that corresponded to the cilia dynein ATPase, five different sets of proteins were common to both cilia and basal bodies. Basal bodies did not contain significant amounts of actin, myosin, or desmin.

By virture of their unique motile properties, the cilia found in the oviduct of a variety of vertebrate organisms play a key role in ovum transport (Halbert et al., 1976; Norwood et al., 1978). Each of the 200–300 cilia in a typical oviduct cell is in continuity with a cortically placed basal body. Although the basal body and the cilium form a functional unit, it is possible to remove the cilia from the cell (Anderson, 1974, 1977) and to extract the basal bodies for purification by cell fractionation procedures (Anderson, 1977; Anderson & Floyd, 1978). The unique biochemical and functional properties of the basal body can then be studied in the isolated organelle.

The basal body as seen within the ciliated oviduct cell (Anderson, 1972) is a complex structure that has several components. The structural core of the organelle is made up of nine sets of three microtubules, each of which is embedded in an electron dense, fibrous meshwork. Thus, the wall of this cylindrical organelle consists of both microtubules and matrix material. Three distinct appendages are attached to the wall of the basal body. To the basal end of the organelle is attached a rootlet structure that consists of longitudinally arranged fibrous elements interrupted at regular intervals by electron-dense cross-striations. Projecting at a right angle from the wall is the basal foot, a pyramid-shaped structure made up of fibrous material. Finally, at the apical end, near the basal body–cilium junction, there are nine sheetlike structures (alar sheets), each of which radiates from one of the nine sets of microtubules. These sheets are positioned such that their apex makes contact with the plasma membrane of the cell. All of these components are also present in the isolated organelle (Anderson, 1974, 1977).

In contrast to the cilium, where the chemistry and function has been studied extensively, very little is known about the basal body. Most of the biochemical studies thus far have utilized impure preparations of basal bodies derived from either protozoan (Rubin & Cunningham, 1973; Wolfe, 1970, 1972) or metazoan sources (Stephens, 1975). One conclusion from these studies is that basal bodies contain tubulin, the major microtubule protein. Several studies have been concerned with the protein composition of the rootlet structure. Stephens (1975) studied the basal apparatus from the scallop gill epithelium, which is rich in rootlets. Using differential extraction techniques, he concluded that the rootlet is composed primarily of two high molecular weight proteins (230 000 and 250 000 daltons). Other rootlet structures may contain a 90 000-dalton protein as the major macromolecule (Amos et al., 1979). Finally, cytochemical studies indicate that basal bodies and basal body rootlets have ATPase activity (Matsusaka, 1967; Nayak, 1972; Nayak & Wu, 1975).

Given the paucity of data on the function and chemistry of the basal body and its homologue the centriole, several years ago this laboratory embarked upon a program to study basic aspects of basal body chemistry. The chicken oviduct basal body was chosen as a source, and methods were developed to isolate this organelle to reasonable purity (Anderson, 1974, 1977; Anderson & Floyd, 1978). When such preparations have been used, it has been possible to demonstrate that oviduct basal bodies contain a unique ATPase (Anderson, 1977) and that this ATPase may be associated with ATP-induced conformational changes detected in suspensions of isolated basal bodies (Anderson & Floyd, 1978). Thus, the isolated oviduct basal body has been useful for deriving biochemical information that may relate to the function of this organelle. To continue further with these studies, we have analyzed the protein composition of this organelle.

Materials and Methods

Materials. Chemicals used in this study include tris(hydroxymethyl)aminoethane hydrochloride (Tris-HCl), lauryl sulfate, agarose, 2-(*N*-morpholino)ethanesulfonic acid (MES), ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), ethylenediaminetetraacetic acid (EDTA), guanosine 5′-triphosphate (GTP), Triton X-100, and dithiothreitol, all of which were from Sigma Chemical Co. (St. Louis, MO). Chemicals used in polyacrylamide gel electrophoresis were from Bio-Rad (Richmond, CA). *N*-(2-Hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid (Hepes) and piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (Pipes) were from Calbiochem (La Jolla, CA). Hank's balanced salt solution (HBSS) was from Grand Island Biological Co. (Grand Island, NY). Nonidet P-40 (NP-40) was from Gallard-Schlesinger Chemical Mfg. Corp. (Carle Place, NY).

Isolation of Basal Bodies. Basal bodies were isolated from chicken oviducts according to previously published procedures (Anderson, 1977). The oviducts were removed from four adult female chickens, cut into small pieces, placed in four 1-L trypsinizing flasks, and washed 4 times in HBSS. The tissue was then agitated in a deciliation medium consisting of 0.25

† From the Department of Cell Biology, The University of Texas Health Science Center at Dallas, Dallas, Texas 75235. *Received April 15, 1980.* This study was supported by a grant from the National Institutes of Health (HD08987).

0006-2960/80/0419-5625$01.00/0

M sucrose, 0.02 M Pipes, pH 5.5, 0.01 M $CaCl_2$, 0.01 M KCl, and 0.05% Triton X-100. The intact epithelial cells remaining after deciliation were detached by agitation in a solution containing 0.25 M sucrose, 0.02 M Hepes, pH 7.5, 0.002 M EDTA, 0.01 M KCl, and 0.025% Triton X-100. From the resulting supernatant fluid, basal bodies were isolated and purified through a series of 1-min sonications in a buffer consisting of 0.02 M Hepes (pH 7.0), 0.01 M KCl, and 0.05% Triton X-100. The final pure fraction of basal bodies was collected from the 1.0–1.5 M interface of a sucrose step gradient after centrifugation for 30 min at 25500*g*. Purified preparations were stored in sucrose at −10 °C.

Electrophoresis. (*1*) *NaDodSO$_4$–Urea Electrophoresis.* Protein preparations were analyzed on NaDodSO$_4$–urea acrylamide gels according to the method of Laemmli (1970). Proteins were dissolved in a solution that contained 8 M urea, 2% NaDodSO$_4$, 5% mercaptoethanol, 10% glycerol, and 0.0625 M Tris-HCl, pH 6.8, at 100 °C for 3 min. The samples were then electrophoresed overnight at a constant voltage of 25 V, using a discontinuous gel system that consisted of a 3% stacking gel and either an 8% separating gel or a linear gradient gel consisting of 4%–16% acrylamide.

(*2*) *NaDodSO$_4$ Electrophoresis.* In some cases, samples were electrophoresed according to the method of Fairbanks et al. (1971). Samples were dissolved in 10 mM Tris-HCl, pH 8, 1 mM EDTA, 1% NaDodSO$_4$, 10% sucrose, and 0.65% dithiothreitol and boiled for 3 min. Samples were then electrophoresed at a constant voltage of 20 V in 7.5% acrylamide gels. All procedures were carried out by using slab gel electrophoresis.

(*3*) *Isoelectric Focusing and Two-Dimensional Electrophoresis.* Samples were isoelectric focused in 3% acrylamide tube gels that contained 2% ampholines (pH 3.5–10 and 5–7 in a ratio of 1:4), 9.5 M urea, and 2% NP-40 (O'Farrell, 1975). For each run, the protein was dissolved in 0.1 mL of lysis buffer consisting of 9.5 M urea, 2% NP-40, 2% ampholines, and 5% mercaptoethanol. Electrophoresis was carried out at 400 V total (eight gels) for 18 h. At completion of the electrophoresis, gels were boiled in 5% Cl_3AcOH for 1 h and then transferred to 1% Cl_3AcOH and allowed to clear overnight. Gels were stained in 0.1% Coomassie brilliant blue R-250 in 50% methanol and 7% acetic acid or 30 min and destained in 7.5% acetic acid for 2 h.

For two-dimensional electrophoresis, the method of O'-Farrell (1975) was used. Samples were isoelectric focused as described above. The tube gels were then numbered and equilibrated in buffer A (10% glycerol, 5% β-mercaptoethanol, 2% NaDodSO$_4$, and 0.0625 M Tris-HCl, pH 6.8). The cylindrical gels were straightened lengthwise and applied horizontally to a 3% stacking gel prepared for slab gel electrophoresis in NaDodSO$_4$–urea. The tube gel was sealed with 1% agarose in buffer A and electrophoresed at a constant voltage of 20 V for 19 h.

(*4*) *Densitometric Analysis.* Coomassie brialliant blue R-250 stained gels were scanned on an ISCO gel scanning device (Gel Scanner, Model 659), using the UA-5 absorbance monitor (Instrument Specialties Co., Lincoln, NE) with a 640-nm filter.

(*5*) *Gel Staining.* Gels were stained with Coomassie brilliant blue R-250.

Peptide Digests. Protease digestion of purified tubulins was carried out according to the method of Cleveland et al. (1977). α- and β-tubulins from either brain microtubules, basal body microtubules, or cilia microtubules were purified by two cycles of electrophoresis. Either basal body, cilia, or brain microtubules were first electrophoresed by using the method of Fairbanks et al. (1971), and the prominent tubulin band from each gel was eluted electrophoretically into a dialysis chamber. The proteins were precipitated with 20% Cl_3AcOH, pelleted, and washed one time with ethyl ether. The pellets were then solubilized in NaDodSO$_4$–urea sample buffer and electrophoresed by using the method of Laemmli (1970). Under these conditions, the prominent tubulin bands were separated into the α and β components. Following brief staining, the α and β bands were cut out of the second gels and eluted by electrophoresis into a dialysis chamber. The eluted proteins were precipitated with 20% Cl_3AcOH and washed with ethyl ether. The second precipitates were resuspended in digestion sample buffer consisting of 0.125 M Tris-HCl, pH 6.8, 0.5% NaDodSO$_4$, glycerol, and 0.1% bromophenol blue. The final concentration of protein was approximately 1 mg/mL in each sample. The samples were divided in half. One half was electrophoresed without digestion, whereas the other half was processed for chymotrypsin digestion. In this procedure, the samples were heated to 100 °C for 2 min, after which 33 μg/mL chymotrypsin was added and the digestion carried out at 37 °C for 30 min. After the digestion period, 10% mercaptoethanol and 2% NaDodSO$_4$, were added, and the samples were boiled for 2 min before electrophoresis. The samples were then subjected to NaDodSO$_4$–urea electrophoresis overnight at a constant voltage of 20 V.

Isolation of Chick Brain Tubulin. Chick brain tubulin was isolated by a modification of the method of Shelanski et al. (1973). Brains from 2-week-old chickens were homogenized in reassembly buffer (0.1 M MES, pH 6.4, 1 mM EGTA, 1 mM GTP, and 0.5 mM $MgCl_2$) at 4 °C in a ratio of 1 g wet weight of brain to 1 mL of buffer. The samples were spun at 43500*g* (4 °C) for 1 h. The pellet was discarded and the supernatant fluid warmed to 25 °C and mixed in a 1:1 ratio with 8 M glycerol. The samples were incubated for 20 min at 37 °C and spun at 43500*g* (25 °C) to sediment the polymerized microtubules. This process was repeated to obtain 2 times, reassembled microtubules.

Results

Basal Body Tubulin. Basal bodies and cilia are a functional unit within the cell and both contain microtubules. Therefore, in the initial electrophoretic analysis, cilia were used as a standard against which to compare basal bodies. Figure 1 is an electrophoretogram that shows the protein patterns for purified oviduct cilia and basal bodies as they appeared after NaDodSO$_4$–urea electrophoresis in tube gels. In addition to numerous bands, the cilia gel contained a high molecular weight complex in the range of 300 000–400 000 (C1) and two prominent bands that migrated in the range of 52 000–56 000 (C2, C3). Whereas the high molecular weight complex most likely was dynein, the cilia ATPase (Gibbons & Rowe, 1965), the doublet bands corresponded to the α and β subunits of tubulin because they had the same electrophoretic mobilities as purified chick brain tubulin that was coelectrophoresed in a companion gel. The basal body gel, by contrast, contained quite a different population of Coomassie blue staining bands. Most notably, a band was present with a molecular weight of ~200 000 (B1), two bands were present (B2, B3) that had a slightly slower mobility than the cilia tubulin subunits, and a prominent band was seen that had a molecular weight of ~17 400 (B4). Because the B2, B3 doublet bands were equal in intensity and because basal bodies are known to contain microtubules, it was assumed that this complex corresponded to the α and β subunits of basal body tubulin. However, under these conditions, this set of bands reproducibly migrated more

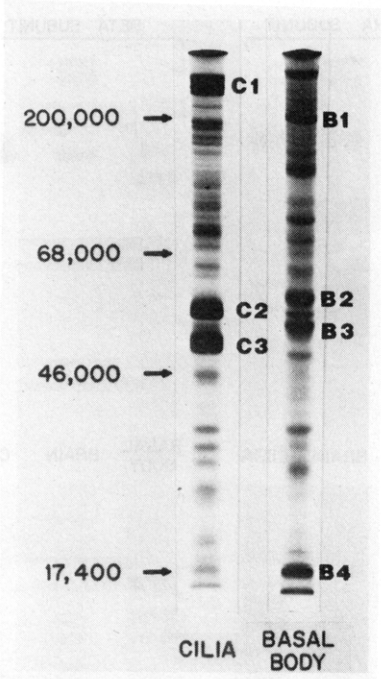

FIGURE 1: $NaDodSO_4$–urea tube gel electrophoresis of purified cilia (100 μg/gel) and purified basal bodies (400 μg/gel). Cilia and basal bodies were isolated from oviducts and prepared for tube gel electrophoresis by solubilization in a solution of 8 M urea, 2% $NaDodSO_4$, 5% mercaptoethanol, 10% glycerol, and 0.0625 M Tris-HCl, pH 6.8, at 100 °C for 3 min. Samples were layered over a 3% stacking gel and electrophoresed at 25 V for 18 h. The running gel was 8% acrylamide. It was found that proportionately larger amounts of basal body protein had to be layered onto the gel to obtain good electrophoretic patterns. Molecular weight markers were myosin (200 000), bovine serum albumin (68 000), actin (46 000), myoglobin (17 400). Both sample gels and gels containing standards were electrophoresed at the same time.

slowly than the cilia tubulin subunits.

It was noticed that when basal bodies were electrophoresed in an $NaDodSO_4$–urea tube gel system, not all of the protein entered the gel. Therefore, other electrophoretic methods were utilized to find conditions that would allow all of the basal body protein to enter the gel. It was found that this could be achieved if the organelles were subjected to slab gel electrophoresis by using either the procedure of Fairbanks et al. (1971) or the procedure of Laemmli (1970). When basal bodies and cilia were subjected to $NaDodSO_4$–urea electrophoresis in a slab gel that contained a linear gradient of acrylamide (4%–16%), prominent doublet bands were present in both gels that had the characteristics of the tubulin subunits (× in Figure 6). Measurements of the mobilities of the doublet bands showed that those in the basal body gel migrated more slowly than those in the cilium gel. In contrast, when these two organelles were processed for $NaDodSO_4$–electrophoresis, the migration pattern of the apparent tubulin band for basal bodies was inconsistent. Most often, a closely spaced doublet band was present in a position that had the approximate mobility of tubulin (arrow, Figure 2A). At other times, only one band was seen in this region of the basal body gel (arrow, Figure 2B). In gels of cilia electrophoresed under identical conditions, only one tubulin band was ever seen (arrow, Figure 2C); however, this band migrated more rapidly than the corresponding band in the basal body pattern.

These electrophoretic patterns established that oviduct cilia contained tubulin, which migrated in $NaDodSO_4$–urea with characteristics that many investigators have reported for this molecule. Basal bodies also appeared to have tubulin, but it clearly behaved differently under several different, one-dimensional electrophoretic conditions. If these bands corresponded to the tubulin subunits, most likely this tubulin had different properties than cilia tubulin. Therefore, a series of electrophoretic experiments was carried out to further explore the nature of the putative basal body tubulin subunits.

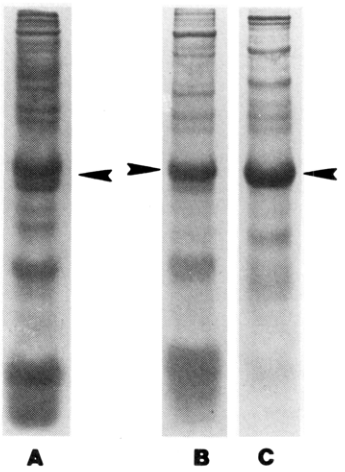

FIGURE 2: Slab gel $NaDodSO_4$ electrophoresis of basal bodies (A, B) and cilia (C). All procedures were as described under Material and Methods. Lane A represents the usual electrophoretic pattern obtained when basal bodies are electrophoresed under these conditions. Typically, the tubulin region of these gels (arrow) contains two bands. Occasionally basal body tubulin migrates as one band in $NaDodSO_4$ electrophoresis (arrow, B); however, the tubulin migrates more slowly than the corresponding molecule in gels of cilia (arrow, C). Lanes B and C are from the same electrophoretic gel.

For a more detailed analysis of the Coomassie blue staining bands seen on $NaDodSO_4$ gels that were thought to correspond to basal body and cilia tubulin, these bands were eluted from gels and subjected to electrophoresis under several different conditions. When these eluted bands were processed for two-dimensional electrophoresis, which involved isoelectric focusing and $NaDodSO_4$–urea electrophoresis, both cilia and basal body proteins behaved similarly (Figure 3A,B). Both sets of proteins migrated in $NaDodSO_4$–urea as a closely spaced doublet (× in Figure 3A,B), and both had approximately the same isoelectric point. The two-dimensional gels of these proteins also indicated that the one-dimensionally purified tubulins were not homogeneous since several, more basic proteins were present in the same molecular weight region as tubulin. This latter observation was substantiated in isoelectric focusing gels of purified basal body tubulin (Figure 4). Here it was seen that the tubulin group had an isoelectric point of 5.45–5.1, and the major, more basic protein (arrow, Figure 3B) had an isoelectric point of 6.73. This set of experiments suggested that the band(s) seen on $NaDodSO_4$ electrophoresis gels of basal bodies represented tubulin.

In another series of experiments, the tubulin subunits from basal bodies, cilia, and brain were purified, separated, and analyzed by $NaDodSO_4$–urea electrophoresis. In the top panel of Figure 5, the α subunits are shown on the left and the β subunits on the right. Within each set of subunits, the brain and cilia components had the same electrophoretic mobility; however, in each case the basal body subunits migrated faster. These results suggested that there was an intrinsic difference in the one-dimensional electrophoretic mobilities of basal body tubulin subunits when compared to cilia tubulin subunits. In other trials, the difference in mobility of the individual subunits was observed; however, as a rule, the difference in mobilities was less than that observed in the experiment shown in Figure 5. Furthermore, when either both α subunits or both β subunits were mixed together in the same well, the two subunits

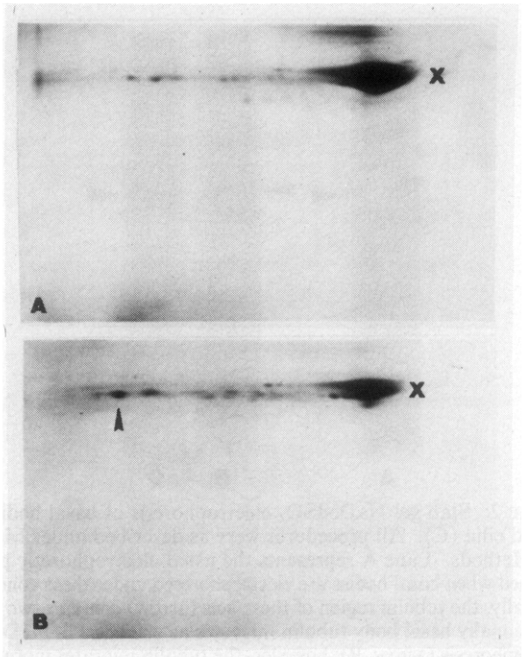

FIGURE 3: Comparison by two-dimensional electrophoresis of tubulins purified from cilia (A) and basal bodies (B). The tubulins were electrophoretically purified on $NaDodSO_4$ electrophoresis slab gels. Each was prepared in the same experimental protocol to ensure identical handling. The purified samples were processed for two-dimensional analysis, as described (see Materials and Methods). The second dimension contained 10% acrylamide. The prominent doublet band (×) corresponds to the α and β subunits of tubulin. Notice that both cilia tubulin and basal body tubulin have nearly identical migration patterns. However, several other protein bands, which have more basic isoelectric points, migrate in the same molecular weight region as tubulin.

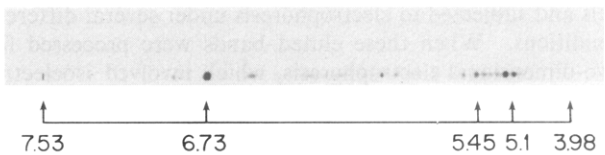

FIGURE 4: Isoelectric focusing pattern of the purified basal body tubulin. Tubulin was purified from whole basal bodies by elution from acrylamide gels, following $NaDodSO_4$ electrophoresis. Protein concentration was adjusted to 1 mg/mL in lysis buffer, and 20 μL of this sample was loaded onto the gel. Focusing was carried out at 400 V for 19.5 h, and gels were stained as described (Materials and Methods). Companion samples were processed for pH determination. Gels were sectioned into 0.5-cm segments and placed in 1 mL of degassed, distilled water for 3 h. The pH for each segment was measured with a Corning pH meter. The range pH 5.45–5.1 indicates the area of tubulin isoelectric focusing, as determined by two-dimensional gels (see Figures 3, 7). This gel demonstrates that electrophoretically purified tubulin contains a mixture of proteins with different isoelectric points. As shown in Figure 3, all of these bands migrate in $NaDodSO_4$–urea within the same molecular weight range as tubulin.

within each set were not seen to separate into individual bands. The lack of an observable separation of the individual subunits most likely was due to the fact that the difference in mobilities was not great enough to resolve the individual subunits when they were mixed together.

A companion set of α and β subunits isolated, as shown in Figure 5, was used for protease digest analysis. The upper panel (Figure 5) shows the half of the subunits that was not digested, and the bottom panel shows the other half that was digested with chymotrypsin before $NaDodSO_4$–urea electrophoresis. Although minor differences were seen between brain and cilia α subunits, they were approximately equivalent. On the other hand, the basal body α subunit had quite a different

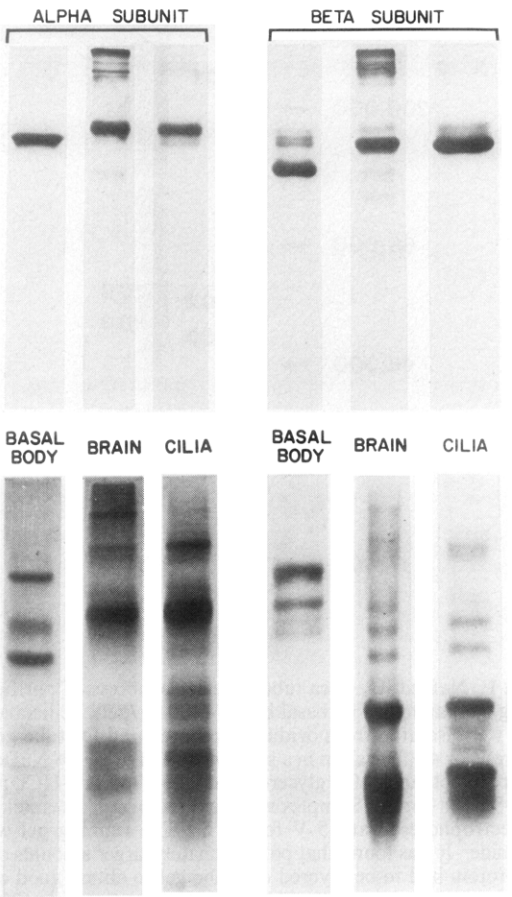

FIGURE 5: Peptide digest of tubulins purified from chicken cilia, chick brain, and chicken basal bodies. The tubulin dimer for each sample was electrophroetically purified, as described in Figure 3 (see Materials and Methods). The purified tubulins were then electrophoresed in $NaDodSO_4$–urea to separate the α and β subunits. These were then eluted from the gel and used in the digestion procedure. Each protein was adjusted to 1 mg/mL in sample buffer (see Materials and Methods) and then divided in half. One half was not treated with protease before $NaDodSO_4$–urea electrophoresis (upper panel), whereas the other half was mixed with 1 μL of chymotrypsin (0.033 mg/mL) and digested before $NaDodSO_4$–urea electrophoresis (lower panel) on a companion gel. Approximately 30 μg of protein was loaded in each sample, and both were electrophoresed in the same slab gel electrophoresis chamber, using 10% acrylamide. The upper panel demonstrates that the proper subunits have been isolated and that each sample contains approximately the same protein concentration. Furthermore, the α and β subunits from basal bodies migrate faster under these conditions than the corresponding subunits for cilia and brain tubulin. The proteolytic digests of the various tubulins (bottom panel) indicate that the basal body tubulin is different from the tubulins for cilia and brain.

digestion pattern. The same was found for the β subunit. The brain and cilia β subunits had almost identical digestion patterns, but the basal body β subunit was distinctive. These differences in the digestion patterns of the tubulin subunits were reproducible since similar results were obtained in three different digestion experiments. Therefore, under conditions where three sources of tubulin were processed in an identical fashion, the basal body tubulin had a unique digestion pattern. These results support the idea that basal body tubulin is different from either cilia tubulin or brain tubulin.

Other Basal Body Proteins. On the basis of the $NaDodSO_4$–urea tube gels shown in Figure 1, it appeared that basal bodies and cilia had very few proteins in common. However, this technique has limited resolving power. Gradient gel $NaDodSO_4$–urea electrophoresis offers much better resolution (see Figure 6). Under these conditions, several sets of proteins

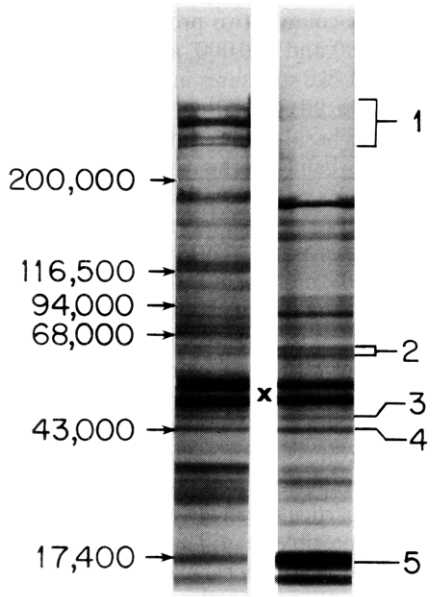

FIGURE 6: $NaDodSO_4$–urea electrophoresis of cilia (left) and basal bodies (right) in gradient acrylamide gels. Approximately 100 μg of protein was dissolved in sample buffer (see Materials and Methods) and layered on a 3% acrylamide stacking gel. The running gel consisted of a 4%–16% linear gradient of acrylamide. Samples were electrophoresed at 25 V for 18 h. × corresponds to the position of the α and β subunits of tubulin. The basal body tubulin subunits appear to migrate more slowly than the corresponding subunits in cilia. These gels establish that under these conditions basal bodies do not contain dynein (region 1) but that common proteins are found in regions 2–5.

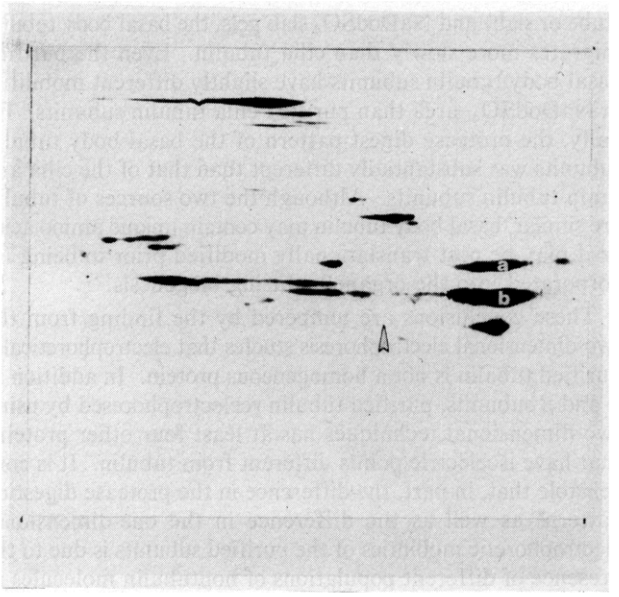

FIGURE 7: Two-dimensional electrophoretic pattern of purified chicken oviduct basal bodies. All procedures were as described under Materials and Methods. In a companion experiment, a preparation of gizzard extract (kindly provided by Dr. Howard Feit) was processed for two-dimensional electrophoresis to determine the migration pattern for actin and desmin. The arrow indicates the position of actin, whereas desmin should be located a little above and to the right of the arrow. This electrophoretogram of basal bodies does not contain bands that correspond to the position for either actin or desmin. (a) and (b) indicate the position for α-tubulin and β-tubulin, respectively. The prominent high molecular weight band does not correspond to myosin (Whalen et al., 1978).

appeared to be common to both organelles. Whereas the two different sets of α- and β-tubulins had different electrophoretic mobilities (based on measurements to the center of each band), bands in regions 2–5 were common to both gels even though the intensity of these bands was different. Also, under these conditions, the prominent high molecular weight band in the basal body gels (B1 in Figure 1) did not comigrate with myosin, which indicates that basal bodies did not contain this protein.

For further examination of the protein composition of basal bodies, the organelles were analyzed by two-dimensional electrophoresis. This technique was used primarily to assess whether or not basal bodies contain either contractile proteins or microfilament proteins. Basal bodies were electrophoresed according to the method of O'Farrell (1975). Companion gels that contained desmin and actin (from chicken gizzard) were electrophoresed to determine where these two proteins should migrate on the basal body gel. Figure 7 shows the results of this experiment. The arrow indicates the approximate location where actin should migrate. Desmin should migrate a little more slowly and should focus at a more acid pH. Since bands were not present in this region, the basal bodies must not have contained substantial amounts of these proteins. The α- and β-tubulin bands were identifiable on the gel (a and b), but the low molecular weight band (~17400) did not enter the isoelectric focusing gel. These gels also showed that the high molecular weight protein (B1, Figure 1) had an isoelectric point that was quite different from myosin (Whalen et al., 1978).

The $NaDodSO_4$–urea tube gel of basal bodies and cilia (Figure 1) suggested that the high molecular weight dynein ATPase complex was not present in isolated basal bodies. Likewise, when basal bodies and cilia were electrophoresed in a gradient gel system (Figure 6), cilia had a high molecular weight complex of several bands that was not present in basal bodies (region 1, Figure 6). Although oviduct cilia dynein has not been purified and characterized, on the basis of studies by several other investigators (Baccetti et al., 1979; Gibbons & Rowe, 1965), the high molecular weight complex seen in Figure 6 most likely contained dynein. Therefore, under these conditions, basal bodies did not contain a detectable amount of dynein.

Discussion

Because basal bodies and cilia are contiguous organelles that presumably have quite different functions and because cilia and flagella have been extensively studied, in the present study the protein profiles of the two organelles have been compared. Two important conclusions from this analysis are that, in addition to tubulin, four groups of proteins appear to be common to the two organelles and that the high molecular weight ATPase in cilia (dynein) is not present in basal bodies.

As one would predict from morphology, tubulin is a major protein in basal bodies respresenting approximately 20% of the total protein. Recalling that microtubules in these organelles occur as triplets rather than as singlets or doublets, it is reasonable to suppose that basal body tubulin might be different from either cilia tubulin or cytoplasmic tubulin and, at the same time, share some common properties. Thus, in an electrophoretic analysis, under some conditions the molecules may behave in an identical way but appear quite distinctive in other types of assays. This is precisely what was found in five different types of electrophoretic experiments. On the one hand, the two sources of tubulin behaved almost identically on two-dimensional electrophoretic gels, which indicates that cilia and basal body tubulin have similar isoelectric points; however, the two sources of tubulin behaved differently in the other electrophoretic experiments. When basal bodies and cilia are electrophoresed on one-dimensional $NaDodSO_4$–urea gels

(tube or slab) and $NaDodSO_4$ slab gels, the basal body tubulin migrates more slowly than cilia tubulin. Even the purified basal body tubulin subunits have slightly different mobilities in $NaDodSO_4$–urea than purified cilia tubulin subunits. Finally, the protease digest pattern of the basal body tubulin subunits was substantially different than that of the cilia and brain tubulin subunits. Although the two sources of tubulin are similar, basal body tubulin may contain unique amino acids or it may be post-translationally modified prior to being incorporated into the organelle during biogenesis.

These conclusions are tempered by the finding from the two-dimensional electrophoresis studies that electrophoretically purified tubulin is not a homogeneous protein. In addition to α and β subunits, purified tubulin reelectrophoresed by using two-dimensional techniques has at least four other proteins that have isoelectric points different from tubulin. It is conceivable that, in part, the difference in the protease digestion patterns as well as the difference in the one-dimensional electrophoretic mobilities of the purified subunits is due to the presence of different populations of nontubulin molecules in the purified preparations of tubulin subunits derived from cilia, brain, and basal bodies.

Tubulin heterogeneity has been demonstrated both within the same cell (Fulton & Simpson, 1976; Stephens, 1970) and between different cellular sources of the protein (Bibring et al., 1976; Feit et al., 1971; Olmsted et al., 1971; Stephens, 1978); however, all tubulins analyzed thus far have the same isoelectric focusing properties (Stephens, 1978). The observation that cytoplasmic tubulin in *Nagleria* is antigenically different from flagellar tubulin [reviewed in Fulton & Simpson (1976)] establishes a precedent that the tubulins from two different organelles within the same cell can be unique.

This laboratory has previously demonstrated, by both biochemical and cytochemical analysis, that basal bodies contain a unique set of ATPases (Anderson, 1977). The conclusion that basal body ATPase was different than cilia ATPase was based on the comparative biochemical behavior of the two sources of enzyme. The present electrophoretic analysis supports that conclusion because the high molecular weight bands (~300 000–350 000 daltons) present in cilia, which are attributable to the cilia ATPase (Gibbons & Rowe, 1965), are not present in one-dimensional gels of basal bodies. Therefore, the basal body ATPase is not dynein-like because the enzyme(s) does not migrate on $NaDodSO_4$–urea gels like dynein and because there are not any morphologically recognizable dynein arms attached to the triplet microtubules (Anderson, 1972; Warner et al., 1977). At the present time it is not known which proteins correspond to the basal body ATPase(s).

Basal bodies may play a role in cell motility (Anderson & Floyd, 1978); furthermore, the rootlet and basal foot contain filamentous components. Therefore, the electrophoretograms were analyzed for the presence of either contractile proteins or other microfilament proteins. A major band was present in one-dimensional tube gels that comigrated with myosin; however, on $NaDodSO_4$–urea gradient gels and on two-dimensional gels, this protein did not coelectrophorese with myosin (Whalen et al., 1978). It was also apparent on two-dimensional gels that neither actin nor desmin was present in the region where they should have migrated had they been present in large concentrations. Therefore, in oviduct basal bodies, the common microfilament proteins are not present in significant quantities.

Isolated chicken oviduct basal bodies have rootlets; therefore, the electrophoretograms of this organelle should contain rootlet structural proteins. Stephens (1975) reported that scallop gill rootlet structures contain two proteins that have molecular weights of 230 000 and 250 000, and Amos et al. (1979) reported that rootlet-like structures in the flagellate *Trichomonas* are composed of a 90 000-dalton protein. Whereas one-dimensional gels of basal bodies did not show any major high molecular weight bands in the range of 230 000–250 000, a prominent band was present with an approximate molecular weight of 90 000 (Figure 6), a molecular weight that is similar to the protein found in the rootlet from *Trichomonas*. It is also possible that the prominent 180 000-dalton protein (Figure 6) in basal bodies could be derived from the rootlet. Further work will be required to positively determine if either of these proteins is a major structural component of the rootlet.

On the basis of these studies, it can be concluded that the distinctive morphology of the basal body is reflected in its unique protein composition. Until immunocytochemical procedures are developed, it will not be possible to identify the proteins within a particular part of the organelle. Nevertheless, it should now be possible to utilize electrophoretic techniques to begin to analyze the relationship of protein synthesis to basal body assembly in ciliogenic cells. The rather complex set of events involved in the assembly of this organelle (Anderson & Hein, 1976) has until now been difficult to investigate. With the protein composition of basal bodies characterized, the spatial and temporal relationships of precursor synthesis to assembly will be an important and accessible area of investigation.

Acknowledgments

We are indebted to Dr. Howard Feit for providing the chicken gizzard actin and desmin. We appreciate the technical assistance of Cheryl Hein. We also thank Drs. W. Snell, H. Feit, and F. Grinnell for their helpful critique of the manuscript.

References

Amos, W. B., Grimstone, A. V., & Rothschild, L. J. (1979) *J. Cell Sci. 34*, 139–164.
Anderson, R. G. W. (1972) *J. Cell Biol. 54*, 246–265.
Anderson, R. G. W. (1974) *J. Cell Biol. 60*, 393–404.
Anderson, R. G. W. (1977) *J. Cell Biol. 74*, 547–560.
Anderson, R. G. W., & Hein, C. E. (1976) *Cell Tissue Res. 171*, 459–466.
Anderson, R. G. W., & Floyd, A. K. (1978) *Cell Biol. Int. Rep. 2*, 487–494.
Baccetti, B., Burrini, A. G., Dallai, R., & Pallini, V. (1979) *J. Cell Biol. 80*, 334–340.
Bibring, T., Baxandall, J., Denslow, S., & Walker, B. (1976) *J. Cell Biol. 69*, 301–312.
Cleveland, D. W., Fischer, S. G., Kirschner, M. W., & Laemmli, U. K. (1977) *J. Biol. Chem. 252*, 1102–1106.
Fairbanks, F., Steck, T. L., & Wallach, D. F. H. (1971) *Biochemistry 10*, 2606–2617.
Feit, H., Slusarek, L., & Shelanski, M. L. (1971) *Proc. Natl. Acad. Sci. U.S.A. 68*, 2028–2031.
Fulton, C., & Simpson, P. A. (1976) *Cold Spring Harbor Conf. Cell Proliferation 3*, 987–1006.
Gibbons, I. R., & Rowe, A. J. (1965) *Science (Washington, D.C.) 149*, 424–425.
Halbert, S. A., Tam, P. Y., & Blandau, R. J. (1976) *Science (Washington, D.C.) 191*, 1052–1053.
Laemmli, U. K. (1970) *Nature (London) 227*, 680–685.
Matsusaka, R. (1967) *J. Cell Biol. 33*, 203–208.
Nayak, R. K. (1972) *J. Histochem. Cytochem. 20*, 840.
Nayak, R. K., & Wu, A. S. H. (1975) *J. Anim. Sci. 41*, 1077–1082.

Norwood, J. T., Hein, C. E., Halbert, S. A., & Anderson, R. G. W. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 4413–4416.
O'Farrell, P. H. (1975) *J. Biol. Chem. 250*, 4007–4021.
Olmsted, J. B., Witman, G. B., Carlson, K., & Rosenbaum, J. L. (1971) *Proc. Natl. Acad. Sci. U.S.A. 68*, 2273–2277.
Rubin, R. W., & Cunningham, W. P. (1973) *J. Cell Biol. 57*, 601–612.
Shelanski, M. L., Gaskin, F., & Cantor, C. R. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 765–768.
Stephens, R. E. (1970) *J. Mol. Biol. 47*, 353–363.
Stephens, R. E. (1975) *J. Cell Biol. 64*, 408–420.
Stephens, R. E. (1978) *Biochemistry 17*, 2882–2891.
Warner, F. D., Mitchell, D. R., & Perkins, C. R. (1977) *J. Mol. Biol. 114*, 307–384.
Whalen, R. G., Butler-Brown, G. S., & Gros, F. (1978) *J. Mol. Biol. 126*, 415–431.
Wolfe, J. (1970) *J. Cell Sci. 6*, 679–700.
Wolfe, J. (1972) *Adv. Cell Mol. Biol. 2*, 151–192.

Molecular Cloning of the Gene Sequences of a Major Apoprotein in Avian Very Low Density Lipoproteins†

Lawrence Chan,* Achilles Dugaiczyk, and Anthony R. Means

ABSTRACT: ApoVLDL-II is a major apoprotein in avian very low density lipoproteins (VLDL). Its synthesis in the cockerel liver is markedly stimulated by estrogen treatment [Chan, L., Jackson, R. L., O'Malley, B. W., & Means, A. R. (1976) *J. Clin. Invest. 58*, 368–379]. We have partially purified apoVLDL-II mRNA from the liver of estrogen-treated cockerels by the following procedures: total nucleic acid extract, oligo(dT)–cellulose chromatography, Sepharose 4B chromatography, repeat oligo(dT)–cellulose chromatography, and sucrose gradient ultracentrifugation. A double-stranded complementary DNA (ds cDNA) was synthesized from the partially (25–30%) pure mRNA and inserted into the *Pst*I site of the plasmid pBR322. Amplification of the chimeric plasmids was accomplished by transformation in *Escherichia coli* RRI strain, and clones were screened by direct colony transfer and in situ hybridization by using the partially pure [^{32}P]-apoVLDL-II cDNA probe. The DNAs of positive clones were isolated and further studied by the hybrid-arrested cell-free translation technique. One clone, pVL10, which inhibited the translation of apoVLDL-II mRNA, was further characterized by DNA partial sequencing. It was found to contain nucleotides which code for amino acids 62–75 of apoVLDL-II. Hybridization of a nick-translated [^{32}P]pVL10 *Hha*I/*Hph*I fragment to the apoVLDL-II mRNA resulted in a $R_0t_{1/2}$ of 5.6×10^{-3} with >90% completion of hybridization. When a similar nick-translated probe was used, apoVLDL-II mRNA sequences were quantified in cockerel liver RNA before and 12 h after a single injection (2 mg) of diethylstilbestrol (DES). Hormone treatment resulted in a 12 000-fold increase in the concentration of apoVLDL-II specific sequences within 12 h after DES. In contrast, such sequences were not detected (up to a R_0t of 3×10^3) in RNA samples isolated from the breast muscles of these animals.

The estrogen-treated cockerel has been used as a model system for investigations into the mechanisms of steroid hormone action as well as the molecular aspects of lipoprotein synthesis. In this animal, estrogen administration markedly stimulates lipoprotein synthesis, involving mainly very low density lipoproteins (VLDL)[1] (Hillyard et al., 1956; Luskey et al., 1974; Chan et al., 1976, 1977). There are two major apoproteins in avian VLDL which account for over 90% of the proteins in VLDL. They have been designated apoVLDL-I and apoVLDL-II. The plasma levels of both proteins are markedly stimulated by estrogen. ApoVLDL-II has been purified to homogeneity, and its primary sequence has been determined (Chan et al., 1976a,b; Jackson et al., 1977). The mRNA for apoVLDL-II has been isolated and its translation product characterized. The latter was found to be larger than the plasma protein by 23 amino acids, containing a highly hydrophobic signal sequence at its amino terminus (Chan et al., 1978, 1980). Estrogen treatment was shown to induce a rapid accumulation of apoVLDL-II mRNA, as demonstrated by in vitro translation assays of avian hepatic mRNA (Chan et al., 1976a, 1978, 1979, 1980).

We now report the purification of the apoVLDL-II structural gene sequence by molecular cloning of the ds cDNA of a partially purified apoVLDL-II mRNA. Conventional techniques of RNA purification were used to prepare an apoVLDL-II-enriched mRNA. Purification of the structural sequences was completed by molecular cloning of the ds cDNA synthesized from the partially purified apoVLDL-II mRNA, using the plasmid pBR322 as vector and *E. coli* strain RRI as host. One of the clones isolated was shown by hybridization analysis, hybrid-arrested cell-free translation, and partial DNA sequencing to contain an inserted apoVLDL-II DNA sequence. Furthermore, using a radiolabeled nick-translated apoVLDL-II DNA probe, we have demonstrated that hepatic apoVLDL-II sequences are markedly stimulated by estrogen.

Materials and Methods

Materials. Four-week-old White Leghorn cockerels were purchased from Animal Specialties Co. Animals received daily injections of diethylstilbestrol (DES), 2.5 mg in sesame oil daily for 1 week, and were sacrificed 24 h after the last injection.

† From the Departments of Cell Biology and Medicine, Baylor College of Medicine, Houston, Texas 77030. *Received April 23, 1980.* This work was supported by National Institutes of Health Grant HL 16512 and American Heart Association Grant 78-1102.

[1] Abbreviations used: VLDL, very low density lipoproteins; EDTA, ethylenediaminetetraacetate; $NaDodSO_4$, sodium dodecyl sulfate; oligo(dT), oligo(thymidylate); DES, diethylstilbestrol; HART, hybrid-arrested cell-free translation; ds cDNA, double-stranded complementary DNA.

0006-2960/80/0419-5631$01.00/0