Dopamine \( \beta \)-hydroxylase is a key enzyme in the biosynthesis of norepinephrine from dopamine. This communication presents amino-terminal sequence data demonstrating that membranous dopamine \( \beta \)-hydroxylase is not anchored by such an uncleaved signal sequence.

EXPERIMENTAL PROCEDURES

Materials—Reagents and chromatographic media were from Sigma, except for the following: electrophoretic reagents were from Bio-Rad, rapid silver stain was from ICN, HPLC grade reagents were from J. T. Baker Chemical Co., water for HPLC analysis was Millipore quality, all sequenator reagents were from Applied Biosystems, and tetrahydrofuran was from Aldrich.

Enzyme Purification—Membranous dopamine \( \beta \)-hydroxylase was purified from fresh bovine adrenal medullae as previously described (Saxena and Fleming, 1983). Purified enzyme was stored in 50 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (Hepes), pH 7.0.

Electrophoretic Elution—The 72 kDa and 75 kDa bands of membranous dopamine \( \beta \)-hydroxylase were electrophoretically eluted from a preparative sodium dodecyl sulfate-polyacrylamide gel and sequenced. Both bands had the amino-terminal sequence characteristic of the soluble bovine enzyme. This sequence results eliminate the possibility that an uncleaved signal sequence serves as the membrane anchor.
Membranous Dopamine β-Hydroxylase NH₂-terminal Sequence

RESULTS AND DISCUSSION

The bovine soluble dopamine β-hydroxylase has previously been shown to possess two amino termini as determined by Skotland et al. (1977) and later modified by Joh and Hwang (1986). The two sequences were of equal stoichiometry and differed only in the presence or absence of the first three amino acid residues (Fig. 1A). The human dopamine β-hydroxylase amino-terminal sequence including the signal sequence has recently been deduced from a cDNA clone (Lamouroux et al., 1987). The predicted human amino terminus contains a 25-residue hydrophobic signal peptide followed by a sequence almost identical to the bovine soluble dopamine β-hydroxylase amino terminus. The first 33 deduced amino-terminal residues for the human enzyme are shown in Fig. 1B with arrows marking the proposed signal peptide cleavage sites. To determine if bovine membranous dopamine β-hydroxylase contained an NH₂-terminal signal sequence similar to that shown in Fig. 1B, purified membranous dopamine β-hydroxylase was subjected to amino-terminal sequence analysis.

The purified bovine dopamine β-hydroxylase used in this study was identical to that described by Saxena and Fleming (1985); it appears to have two major bands of 72 and 75 kDa on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (data not shown). Edman degradation of this purified bovine membranous dopamine β-hydroxylase gave two sequences with approximately equal stoichiometry and a third sequence in lower yields (Table I). Whereas soluble dopamine β-hydroxylase has a low reactivity with the Edman reagent and thus has been suggested to have a partially blocked amino terminus (Skotland et al., 1977; Hogue-Angeletti, 1976), membranous dopamine β-hydroxylase appears to be unblocked as indicated by a high yield of phenylthiohydantoin derivatives shown in Table I (approximately 70% initial yield assuming three different polypeptide chains as described below). Since only five cycles of membranous dopamine β-hydroxylase were analyzed, sequenator performance was evaluated using the sequenator standard β-lactoglobulin. Our calculated average initial and repetitive yields for 1 nmol of protein were 57 and 92%, respectively.

We interpret our data in Table I to be consistent with the consensus sequence for soluble dopamine β-hydroxylase shown in Fig. 1A except that our data show an additional third sequence present at approximately 50% of the other two. This third sequence is completely similar to residues 23-27 of the human sequence shown in Fig. 1B and would result from signal peptide cleavage at the analogous position indicated by the asterisk. No methionine, arginine, or lysine was observed in the first five cycles as might be expected for a typical signal sequence (von Heijne, 1985). The first cycle did contain glycine (944 pmol) which we attribute to contamination because a fourth sequence was not observed in subsequent cycles; and cycle five contained carry-over of proline from cycle four. In cycle three a serine was not identified as expected for position 6 of the consensus sequence shown in Fig. 1A. Instead a peak with the same retention time as the valine phenylthiohydantoin derivative was present in this cycle. However, because Polybrene can produce an artifact at this retention time (Klapper et al., 1988) and taking into consideration the lability of serine during sequencing reactions we have not assigned this residue. The sequences obtained indicate that the complete enzyme does not have an uncleaved hydrophobic signal sequence which can serve as a membrane anchor.

A possible interpretation of the above results was that our amino-terminal sequences for membranous dopamine β-hydroxylase resemble the consensus sequences for the soluble enzyme but now only the 72-kDa subunit is undergoing Edman degradation and that the 75-kDa subunit contained a blocked signal sequence. The high yields of amino acids from the sequenator argue against only half of the subunits having been sequenced. However, we examined this possibility by separating the 72- and 75-kDa bands using electrophoretic elution and subjecting each to 5 cycles of amino-terminal sequence analysis. Each eluted band was free of other detectable subunits when analyzed by SDS-polyacrylamide gel electrophoresis and silver-stained (data not shown). The amino-terminal sequences for the 72- and 75-kDa bands are shown in Table II.

In the sequence analysis of the electroeluted 72-kDa band the expected serine residue in position one could not be identified. The two residues found in cycle one corresponded to alanine and an unknown residue of similar peak area eluting just after alanine. This residue could be dehydroalanine, a breakdown product of serine. Also, the expected serine residue in cycle three could not be identified; only glycine was observed in this cycle. This again could be due to the labile nature of serine residues and the contamination by glycine in the electroeluted samples. Despite these individual differences from the consensus sequence shown in Fig. 1A, both sequences obtained for the 72-kDa band are consistent with the sequences reported for soluble bovine dopamine β-hydroxylase (Table I).

The 75-kDa band also yielded two sequences of approximately equal stoichiometry although no amino acid assignments could be made in cycle one. The second residue in cycle three was not assigned. No serine residue could be identified in this cycle which could be due to the labile nature of serine residues. The only other significant peak in this cycle again had a retention time similar to valine phenylthiohydantoin and may have been due to a Polybrene contaminant which has been observed to elute at this retention time (Klapper et al., 1978). A third sequence could not be identified in either the 72- or 75-kDa sample. If a third sequence were present in these bands in the same proportion as shown in Table I it would have been at the limit of sensitivity for our system.

In summary, the major finding of this work is that membranous bovine dopamine β-hydroxylase does not contain a
but rather to post-translational differences. In addition, some evidence is presented that two major subunits of the membranous hydroxylase are not completely similar to the human hydroxylase sequence. These results indicate that the amino acid sequences of the two major subunits of the membranous hydroxylase are not significantly different in agreement with other types of evidence. In rat tissue or cultured cells, the lower molecular weight subunit is derived from the higher molecular weight binding segment. In addition, some evidence is presented that the amino acid sequences of the two major subunits is glycosylation. Our observations on this topic are in identical polypeptides. Although carbohydrates alone could not be the membrane anchor it is possible that they are instrumental in binding a separate moiety which is hydrophobic. The hydrophobicity of tightly associated phosphatidylinerine found with purified membranous hydroxylase (Saxena and Fleming, 1985) could account for the membrane binding of this form of the enzyme. We have not been able to dissociate this phospholipid from the native form of the enzyme to determine if the lipid itself is the membrane anchor. A direct analysis of the hydrophobic component(s) of the membranous form may be necessary to elucidate the nature of this membrane anchor.

Acknowledgment—We gratefully acknowledge the help of Dr. Carolyn Harley during this work.

REFERENCES
Lamouroux, A., Vigny, A., Faucon Biguet, A., et al. (1983b); only a single molecular weight species of hydroxylase is produced from in vitro translation of total adrenal medulla mRNA (Sabbah et al., 1983a); and nuclease protection experiments with human adrenal medulla RNA indicate only one species of mRNA (Lamouroux et al., 1987). Thus, the membrane binding ability of the 72-kDa subunit (Dhawan et al., 1987) is not likely to be due to a different amino acid composition than the 72-kDa subunit but rather to post-translational differences.

One obvious post-translational modification that could be responsible for the difference between the 75- and 72-kDa subunits is glycosylation. Our observations on this topic are contradictory. In PC12 cells endoglycosidase treatment of the membranous and soluble forms of hydroxylase clearly shows that membranous hydroxylase has a different resistance to endoglycosidase H and endoglycosidase F than soluble hydroxylase (McHugh, 1984). In contrast, endoglycosidase treatment of the two forms of purified bovine hydroxylase results

### Table I

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Membranous*</th>
<th>Soluble</th>
<th>Consensus*</th>
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<td>Leu (980)</td>
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</tr>
<tr>
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<td>Pro (1192)</td>
<td>X</td>
<td>Cys (577)</td>
</tr>
<tr>
<td>4</td>
<td>Ala (865)</td>
<td>Pro (1823)</td>
<td>X</td>
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<tr>
<td>5</td>
<td>Glu (1084)</td>
<td>Phe (813)</td>
<td>Ala (497)</td>
</tr>
</tbody>
</table>

* Purified as described under "Experimental Procedures."

AA, amino acid.

Mr. McHugh gratefully acknowledges the help of Dr. Carolyn Harley during this work.


**Table II**

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Membranous dopamine β-hydroxylase amino-terminal sequence</th>
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* Electrophoretically eluted from SDS-polyacrylamide gel electrophoresis as described under "Experimental Procedures."