The effects of iron deficiency on the NADH- and succinate-oxidizing complexes of rat skeletal muscle mitochondria have been investigated. Both systems were similarly affected: activities were about 30% of normal in dehydrogenase, ubiquinone reductase, and oxidase assays, and similar reductions in the concentration of their respective flavin prosthetic groups were also evident in the iron-deficient membranes. Thus, the turnover numbers of the two enzymes were unchanged in iron deficiency. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis revealed similarly reduced levels of those peptide components of Complexes I and II that could be unequivocally distinguished.

Soluble beef heart succinate dehydrogenase added to alkaline-treated rat skeletal muscle mitochondrial membranes attached to binding sites exposed by the treatment, forming a hybrid complex indistinguishable from the original skeletal muscle complex, with restoration of succinoxidase and succinate-ubiquinone reductase activities to the levels observed in the original rat membranes. Iron-deficient particles behaved like the normal in these tests. No unfilled binding sites for the enzyme could be detected prior to alkaline treatment.

The data are interpreted as indicating that the lower activities of these two respiratory complexes in iron deficiency are due to lower content of the enzymes rather than to the presence of impaired enzymes in the membrane, that only fully competent complexes are present in these membranes, and that iron-deficient complexes are either not assembled or are lost after assembly.

Iron deficiency is recognized as a powerful method for altering the iron content of proteins in mammalian systems (1–3). Of tissues that have been investigated, rat skeletal muscle proved particularly sensitive to dietary iron deficiency (1, 2, 4), showing a dramatic decrease in the activity of mitochondrial respiratory chain dehydrogenases (2, 3, 5). The selective nature of this effect was apparent from the fact that under conditions where both NADH and succinate dehydrogenase activities decreased by 70%, the specific contents of individual cytochromes decreased less than 40%, the amount of Rieske protein declined about 20%, and the specific activity of the ATPase increased slightly (5). In accord with the lower activities of iron-deficient membranes, appropriately smaller EPR signals of the iron-sulfur clusters of NADH and succinate dehydrogenases were observed (5, 6).

Questions that have appeared of interest to us concern the possible presence in iron-deficient membranes of catalytically defective dehydrogenase molecules, and, where the amount of enzyme in the membrane is abnormally low, whether a surfeit of unoccupied enzyme-binding sites might be present in the membrane. Furthermore, in these respects, are the effects of iron deficiency similar for all the iron-containing mitochondrial dehydrogenases? Is a common control mechanism in membrane assembly implied? In the present studies, we have addressed some of these questions using enzymological and analytical approaches to assess the effects of iron deficiency on the non-heme iron centers of the succinate- and NADH-ubiquinone oxidoreductases of rat skeletal muscle mitochondria and to test for free binding sites for succinate dehydrogenase in iron-deficient membranes.

In Complex II, the succinate-ubiquinone oxidoreductase, two hydrophobic peptides, designated as C1313 and C1414 (7), have been identified as integral functional components in addition to succinate dehydrogenase itself (8). It is worth noting that the function or presence of these peptides may also be affected by iron deficiency, since preparations of pure C1313 + C1414 contain b-type cytochrome (8, 9). Their presence is essential for binding succinate dehydrogenase to the membrane, for stabilization of the ubisemiquinone species (10) generated by single electron transfer from the iron-sulfur cluster 31 of the enzyme (10, 11), for formation of the binding site for the specific inhibitor TTF (8, 12), and for protection of cluster 3, which is extremely sensitive to oxygen in soluble enzyme preparations (13, 14). The occurrence of TTF-sensitive ubiquinone reduction is thus a direct indicator of intact, active complexes of succinate dehydrogenase and peptides, and the generation of this activity on the addition of soluble succinate dehydrogenase to mitochondrial membrane preparations indicates the existence of free, active binding sites for the enzyme.

† Supported by National Institutes of Health Grant HL-16251 and the Veterans Administration.
‡ Supported by the National Foundation for Cancer Research and National Institutes of Health.
§ Supported by National Institutes of Health Grant AM-13897.

The abbreviations used are: cluster 3, the paramagnetic oxidized iron-sulfur cluster of succinate dehydrogenase, also referred to as S-3; TTF, thienoyltrifluoroacetone; SMP, submitochondrial particles; PMS, phenazine methosulfate Q, ubiquinone; DCIP, 2,6-dichlorophenol; DPB, 2,3-dimethoxy-5-methyl-6-pentyl-1,4-benzoquinone; SDS, sodium dodecyl sulfate.
Results

The experiment reported in Table I was carried out in efforts to define more precisely the effects of iron deficiency on mitochondrial function, in extension of previous work. The data documented include succinate-PMS reductase activity, which is taken as a measure of both modified and unmodified succinate dehydrogenase activity, and succinate-DPB reductase activity, which measures membrane-bound, unmodified enzyme. In addition, in order to determine whether nonfunctional enzyme was also present, the amount of enzyme in the membrane was estimated by the activity-independent determination of the covariantly bound prosthetic group 8a-histidyl FAD. Approximately 70% decline in both activity and histidyl flavin content was observed, so the catalytic turnover/enzyme molecule was the same in both normal and iron-deficient rat mitochondrial membranes. Thus, all enzyme remaining in the iron-deficient membrane was unmodified, and enzyme simply depleted in Fe/S clusters was not present. A change in the K_m for PMS (~0.22 mM), reported to accompany the decreased activity of iron-deficient membranes (5), and to indicate, possibly, a modified form of the enzyme, was not evident in the present studies.

The effects of iron deficiency on the NADH-Q oxidoreductase (Complex I) segment of the respiratory chain were determined with rotenone-insensitive reduction of ferricyanide as a monitor (23) of the primary dehydrogenase (20), and reduction of DPB for functionality of the remainder of the complex. In assays of NADPH-DPB reductase a range of DPB concentrations (3-40 μM) was used which did not inhibit the reaction; the activity was rotenone-sensitive (>90% inhibition with 3 μM rotenone) and thus representative of electron transport through the NADPH-Q reductase segment of the respiratory chain. The reduction of DPB in both normal and iron-deficient membranes was 14-fold slower than reduction of ferricyanide by the primary dehydrogenase.

The results of these assays, presented in Table II, show

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that iron deficiency produced identical decreases in the NADH-ferricyanide and NADH-DPB reductase activities and in the NADH dehydrogenase content of the membrane, equated here with the content of noncovalently bound FMN. It is of interest that the extent of this decrease matched that occurring in the activity and content of succinate dehydrogenase in the same particle preparation (cf. Table I). A lowering of the $K_m$ for ferricyanide from 1.96 to 0.74 mM accompanied the decreased NADH-ferricyanide reductase activity of iron-deficient membranes, in accord with the results of Maguire et al. (5). This change appears unrelated to catalytic efficiency, since the activity was the same at infinite ferricyanide concentration (turnover number $= 2.7-3.0 \times 10^5$ min$^{-1}$) in both iron-deficient and normal membranes. Iron deficiency had no effect on the $K_m$ for DPB or the turnover number ($2.3 \times 10^4$ min$^{-1}$) in the NADH-DPB reductase assay. Hence, the decreased levels of NADH oxidation observed with iron-deficient membranes were most likely a consequence of less membrane-bound NADH dehydrogenase, although the evidence was less conclusive than with succinate dehydrogenase because of possible losses of the noncovalently attached FMN.

Further evidence for decreased levels of Complexes I and II in iron-deficient membranes was obtained by comparison of the relative staining intensities of the subunits of iron-deficient and normal membranes following SDS-polyacrylamide gel electrophoresis (Fig. 1). Purified Complexes I and II, and the low molecular weight form of NADH dehydrogenase from beef heart served as reference markers. It is clear from the gel traces that the specific content of the 70-kDa subunit of succinate dehydrogenase, which could be identified both by $R_f$ and by the fluorescence of its covalently bound FAD moiety at acid pH, was lower in iron-deficient membranes, in accord with the lower histidyl FAD value established by direct analyses (Table I). Decreases in the contents of peptides migrating in the vicinity of the 27-kDa subunit of succinate dehydrogenase and peptides CII.3 and CII.4, are also apparent, but cannot be unequivocally assigned to the Complex II peptides because of inadequate resolution on the gels and the possibility that the overlapping peptide bands from other complexes might also be subject to iron limitation. Similar considerations pre-

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**Fig. 1.** Effect of iron deficiency on the peptide contents of submitochondrial particles from rat skeletal muscle. Gel scans (A) were obtained of normal (-----) and iron-deficient (----) SMP following SDS-polyacrylamide gel electrophoresis (40-μg aliquots of protein) and staining of the peptide bands as described under "Experimental Procedures." Complex I (15 μg), the "low molecular weight form" of NADH dehydrogenase (7 μg), and Complex II (5 μg), all from beef heart, were similarly analyzed (scans B, C, and D, respectively) for comparison and identification of certain of the rat peptides. Molecular weight markers (scan E) were: i, phosphorylase B (92,500 kDa); ii, bovine serum albumin (66,200 kDa); iii, ovalbumin (45,000 kDa); iv, carboxy-anhydrase (31,000 kDa); v, soybean trypsin inhibitor (21,500 kDa); vi, lysozyme (14,400 kDa).
vented direct assessment of the effects of iron deficiency on the three subunits constituting the low molecular weight form of NADH dehydrogenase, but certain of the other subunits of Complex I could be distinguished and showed less intense staining than the same peptide bands of normal membranes. These included the peptides of 75 and 40 kDa and, less clearly, those in the 14–21-kDa region of the gels.

Since the relative amounts of peptides C_{11.3} and C_{12.4} could not be unequivocally determined on the gels, use was made of the recently defined roles of these peptides in Q reduction to determine if the peptides were present in excess in iron-deficient membranes, where the enzyme concentration was 70% lower than in normal membranes (Table I). For this purpose normal and iron-deficient membranes were examined in reconstitution experiments with purified succinate dehydrogenase from beef heart. To be certain that using the beef heart enzyme was a valid method for detecting binding sites in the rat membranes, the binding sites already occupied by the endogenous dehydrogenase were exposed by incubating the membrane suspensions aerobically at 38 °C and pH 9.3, in the absence of succinate, according to the method developed for treating SMP of beef heart mitochondria (30). Availability of binding sites was monitored by assaying for succinate-DPB reductase activity in both untreated and treated membranes, before and after the addition of an excess of the soluble enzyme. The capacity of the respiratory chain for accepting electrons from membrane-bound succinate dehydrogenase was not impaired by iron deficiency (Table III).

Alkaline treatment of both the normal and deficient membranes resulted in increased levels of succinate-DPB oxidoreductase activity before and after the addition of soluble beef heart succinate dehydrogenase.

**TABLE III**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Normal SMP</th>
<th>Iron-deficient SMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Succinate, 20 mM</td>
<td>0.43</td>
<td>0.16</td>
</tr>
<tr>
<td>NADH, 1 mM</td>
<td>0.83</td>
<td>0.28</td>
</tr>
<tr>
<td>Succinate, 20 mM + NADH, 1 mM</td>
<td>1.16</td>
<td>0.45</td>
</tr>
</tbody>
</table>

**Fig. 2.** Alkaline treatment of submitochondrial particles of normal rat skeletal muscle. SMP (10 mg of protein/ml) were incubated at pH 9.3 at 38 °C as described under "Experimental Procedures," and aliquots were removed at periodic time intervals for assays of activities. A, succinate-DPB oxidoreductase activity before (O) and after (■) the addition of soluble beef heart succinate dehydrogenase. B, succinoxidase activity before (○) and after (□) addition of beef heart succinate dehydrogenase. Succinate-DPB oxidoreductase activity was monitored at a single concentration of DPB (67 μM), with DCIP (52 μM) as terminal electron acceptor.

**Fig. 3.** Alkaline treatment of submitochondrial particles of iron-deficient rat skeletal muscle. Iron-deficient SMP were incubated and assayed as described for normal SMP in the legend to Fig. 2. Succinate-DPB oxidoreductase activity is shown before (○) and after (■) addition of soluble beef heart succinate dehydrogenase.

**Fig. 4.** Effect of alkaline treatment at 30 °C on normal rat skeletal muscle submitochondrial particles. SMP (10 mg of protein/ml) were incubated at pH 9.3 at 30 °C and monitored periodically (see "Experimental Procedures") for NADH oxidase activity (□), succinate-PMS oxidoreductase activity (○), succinate-DPB oxidoreductase activity (△), and Fe/S cluster S-3 of succinate dehydrogenase (■). Succinate-PMS and succinate-DPB reductase activities were measured at single concentrations of PMS (1.08 mM) and DPB (67 μM), respectively, with DCIP (52 μM) as terminal electron acceptor. The amount of cluster S-3 was estimated by measuring the derivative peak height of the feature centered around g = 2.01 in the EPR spectrum. The EPR spectrum had a temperature-sensitive feature which was not detectable above 20 K; this feature of the spectrum was used for calculating the values shown, as it corresponds best to cluster S-3 from rat muscle mitochondria.

branes resulted in rapid, simultaneous loss of endogenous succinoxidase and succinate-DPB reductase activities, which occurred as apparent first order reactions with the same rate constant (k = 0.2 min⁻¹ at 38 °C), in both types of particle. The loss of both activities by normal membranes is shown in Fig. 2 and the loss of succinate-DPB reductase activity by iron-deficient membranes in Fig. 3. NADH oxidase activity was also lost, but at a lower rate (k = 0.08 min⁻¹) than succinate-DPB reductase activity. Addition of reconstitutively active beef heart succinate dehydrogenase to the suspensions of treated membranes resulted in increased levels of succinate-DPB reductase (upper curves, Figs. 2A and 3) and succinoxidase (Fig. 2B) activities, which were fully TTF-sensitive and unaffected by washing of the membranes, and
hence were a consequence of formation of hybrid complexes between "fresh" beef enzyme and exposed binding sites on the rat membranes. No free, active binding sites for succinate dehydrogenase appeared to be present on either normal or iron-deficient membranes, since no increase in membrane-bound activities occurred on adding soluble enzyme to untreated membrane samples (zero time values, Figs. 2 and 3). This was of particular interest in the case of iron-deficient membranes, which contained 70% less enzyme than normal membranes (Table I) and possibly could have contained empty binding sites.

Whereas exposed binding sites were found to be stable at neutral pH, it is evident from Figs. 2 and 3 that at pH 9.3 the capacity of membranes to recombine with the beef heart enzyme declined with time of exposure (Figs. 2 and 3). Whether this relates to in situ inactivation of binding peptides, presumably C14,4 and C14,6, or to their selective solubilization is not yet known. The gradual loss of reconstitutive capacity observed here cannot be ascribed to a general disintegration of the membrane structure at pH 9.3, since it also occurs during alkaline treatment at 30 °C when NADH oxidase activity, and hence integrity of the electron transport system, is conserved (Fig. 4). In this respect, inner mitochondrial membranes of rat skeletal muscle differ from those of beef heart, where the reconstitutive capacity is stable for at least 1 h under similar conditions, pH 9.3 (38 °C) (30). Inactivation of the endogenous succinate-DPB reductase and succinoxidase (not shown) activities still took place at 30 °C and was shown to occur in parallel with the loss of the EPR signal attributable to Fe/S cluster 3 of succinate dehydrogenase and ~50% of the succinate-PMS reductase activity of the suspension.

Table IV provides further details of the consequences of alkaline treatment (10 min at pH 9.3, 38 °C) of normal and iron-deficient membranes and their subsequent recombination with soluble enzyme. Although alkaline treatment of 10 min duration was sufficient to inactivate completely the succinate-DPB reductase activity of the membrane preparations (Figs. 2 and 3) and, concomitantly, cluster 3 of the enzyme (Fig. 4), it is apparent that full dissociation of endogenous enzyme was not achieved. Some 20% of the enzyme in iron-deficient membranes and 30% in normal membranes remained membrane-associated and retained activity in the succinate-PMS reductase assay, albeit with a lower turnover number (6000–7000 min−1) (Table IV, lines 2 and 6). This level of activity was also exhibited by that fraction of the enzyme which had already dissociated from the treated membranes. Neither the residual binding enzyme nor the solubilized form were affected by TTF. It is not known whether the residual enzyme is still associated with binding sites or dislocated sufficiently to expose the binding sites for recombination with fresh enzyme, as appears to happen with beef heart membranes (31). Recombination of "new" soluble enzyme with free binding sites resulted in 60–70% recovery of the succinate-DPB reductase activity exhibited by untreated iron-deficient and normal membranes and a commensurate increase in succinate-PMS reductase activity (lines 3 and 7). The catalytic properties of the newly attached beef heart enzyme were apparently the same as those of the native rat enzyme, as evidenced by the same turnover numbers in the succinate-DPB and succinate-PMS reductase assays as originally observed (cf. lines 1 and 4, and 5 and 8, respectively, for normal and Fe-deficient membranes).

**DISCUSSION**

In the studies reported here the decreased NADH and succinate dehydrogenase activities exhibited by submitochondrial particles from iron-deficient rat skeletal muscle have been reaffirmed and shown to be a consequence of lower levels of these enzymes in the membranes, both from direct analyses for their respective prosthetic groups, noncovalently bound FMN and histidyl FAD, and by the less intense staining of enzyme subunits identifiable in SDS-polyacrylamide gels of iron-deficient membranes. Thus, the NADH and succinate dehydrogenases that are present in iron-deficient membranes reduce artificial electron acceptors and Q with the same turnover numbers as their counterparts in normal membranes, and hence must be fully functional with their essential constituent non-heme iron clusters intact. This information extends earlier EPR data (5, 6) by showing that the smaller signals elicited for non-heme iron clusters in such membranes are due to less membrane-bound enzyme and not to the presence of cluster-deficient enzymes, a previously considered possibility (5). Fully competent Q reductase activity, a membrane-associated function, also necessarily verifies the integrity of those peptides and non-heme iron proteins associated with the succinate and NADH dehydrogenase molecules, respectively, as parts of the succinate-Q and NADH-Q oxi-

<table>
<thead>
<tr>
<th>Sample</th>
<th>Histidyl FAD</th>
<th>PMS reductase</th>
<th>Turnover number</th>
<th>DPB reductase</th>
<th>Turnover number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Normal SMP</td>
<td>0.13</td>
<td>1.92</td>
<td>14,800</td>
<td>1.92</td>
<td>14,800</td>
</tr>
<tr>
<td>2. Same, after pH 9.3 treatment</td>
<td>0.05</td>
<td>0.40</td>
<td>8,000</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>3. pH 9.3-treated + beef heart SDH*</td>
<td>0.13</td>
<td>1.55</td>
<td>11,900</td>
<td>1.20</td>
<td>9,200</td>
</tr>
<tr>
<td>4. Line 3 minus line 2</td>
<td>0.08</td>
<td>1.15</td>
<td>14,400</td>
<td>1.20</td>
<td>15,000</td>
</tr>
<tr>
<td>5. Iron-deficient SMP</td>
<td>0.05</td>
<td>0.77</td>
<td>15,400</td>
<td>0.77</td>
<td>15,400</td>
</tr>
<tr>
<td>6. Same, after pH 9.3 treatment</td>
<td>0.01</td>
<td>0.06</td>
<td>6,000</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>7. pH 9.3-treated + beef heart SDH*</td>
<td>0.04</td>
<td>0.55</td>
<td>13,800</td>
<td>0.51</td>
<td>12,800</td>
</tr>
<tr>
<td>8. Line 7 minus line 6</td>
<td>0.03</td>
<td>0.49</td>
<td>16,300</td>
<td>0.51</td>
<td>17,000</td>
</tr>
</tbody>
</table>

* See footnote to Table I.

* SDH, soluble succinate dehydrogenase.
reductase complexes. These components are assumed to be similar in the rat skeletal muscle mitochondria to those in the respective complexes of beef heart mitochondria, that is, peptides CII.3 and CII.4, which are required for the membrane-bound activities of succinate dehydrogenase (8, 29), and a total of 8–12 peptides and non-heme iron proteins (32, 33) associated with the three subunits comprising the primary NADH dehydrogenase unit (20). It is significant, therefore, that the two most clearly discernible Complex I subunits on the gels shown in Fig. 1, identified by molecular masses of 75 and 40 kDa (32, 33), which are not parts of the primary dehydrogenase, are also in lower concentration in the iron-deficient membranes than in the normal. From the relative staining intensities of the 40-kDa subunit, which is fully resolved in the gels, it can be estimated that this subunit suffers a 60–70% decrease in concentration, close to that observed for the FMN content of the primary dehydrogenase. It is also of interest that this subunit is possibly not a non-heme iron protein, inasmuch as the clusters of Complex I appear to be distributed among others of the subunits (34, 35). The data could imply then that the all subunits of Complex I are adversely affected by iron deficiency, whether or not they contain non-heme iron. This would be compatible with the presence of fewer, but intact, complexes in such membranes.

The limited contents of NADH and succinate dehydrogenases demonstrated here for iron-deficient membranes could reflect those fractions of the enzymes that have received their full complement of non-heme iron, without which binding to the membrane might be absent or transitory. Possibly introduction of non-heme iron clusters into the enzymes occurs prior to assembly in the membrane; this would be consistent with recent findings that succinate dehydrogenase containing EPR-detected non-heme iron can be found in the cytoplasm of Bacillus subtilis mutants unable to complete assembly of the complex in the membrane.2 A role for the membrane as well is nevertheless anticipated in the synthesis or organization of cluster 3, since this Fe/S cluster is extremely labile in soluble succinate dehydrogenase, but is stabilized when buried in the inner mitochondrial membrane (13, 14). The possibility remains that the availability of competent iron-containing subunits is critical for assembly of the other subunits, or that coordinated repression of synthesis of the subunits occurs in iron limitation. This could explain the decreased levels of the 40-kDa peptide of Complex I in iron deficiency, if the peptide indeed does not contain iron.

The absence of excess binding sites able to bind succinate dehydrogenase in iron-deficient membranes containing 70% less enzyme than normal membranes (Figs. 2 and 3) is also consistent with a coordinated decrease of Complex II subunits. The supply of peptides CII.3 and CII.4, however, could well be limiting for the attachment of succinate dehydrogenase to the membrane. One of these, presumably CII.3, is cytochrome b570, the heme of which could be depleted in iron deficiency. Unlike the b-type cytochromes of Complex III, which are of mitochondrial origin (36), cytochrome b570 is cytoplasmically synthesized, according to studies with Neurospora crassa (37), and must be transported to the inner mitochondrial membrane for assembly, where insertion of heme into the peptide may be a required step for its processing to final form, as with cytochrome c1 in yeast (38). The dehydrogenase content of the rat membranes could then reflect limited availability of binding peptides, which because of lack of heme in iron deficiency, could not be processed into a form capable of binding the enzyme. The presence of both active (30%) and inactive (70%) forms of CII.3 and CII.4 in the iron-deficient membranes could not be determined by SDS-polyacylamide gel electrophoresis and remains, therefore, an open question. The situation created in iron-deficient rat skeletal muscle mitochondria might thus be analogous to those occurring in heme-less mutants of B. subtilis, where succinate dehydrogenase remains in the cytoplasm until synthesis of cytochrome b is elicited by addition of l-aminolevulinic (39), and of yeast, where inefficient uptake and assembly of subunits of Complex III (40) and cytochrome oxidase (41) are evident when mature cytochrome c1 and heme a, respectively, are lacking. An alternative explanation for the apparent absence of free binding sites in iron-deficient rat membranes, where the content of succinate dehydrogenase was 70% less than that of normal membranes, is that unfulfilled binding sites are proteolysed. It is known that considerable protection against chymotryptic attack is afforded CII.3 and CII.4 by association with the dehydrogenase in Complex II preparations, whereas in the absence of enzyme the peptides are rapidly hydrolyzed (8). Presumably similar protection, or greater, would be afforded the peptides in submitochondrial particles.

Application to the rat system of the alkaline-treatment method used to prepare beef heart SMP for reconstitution experiments with soluble succinate dehydrogenase showed two major differences from the beef heart system, namely, that concurrently with loss of oxidase activity most of the dehydrogenase could be recombined with exposed enzyme binding sites in the rat skeletal muscle mitochondrial membranes to give stable, hybrid complexes with the same catalytic properties as the native rat complex, i.e. the same turnover number in the PMS and Q reductase assays, and sensitivity to TTF. Since previous work with beef heart Complex II has shown that the correct architecture for the TTF-binding site requires contributions from both succinate dehydrogenase and peptides CII.3 and CII.4, and that specific TTF binding is absent if either component is missing (12), these data strongly suggest that peptides CII.3 and CII.4 are present also in rat membranes, or peptides with significant homology.

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