Enzyme Activity of Macrophage Migration Inhibitory Factor toward Oxidized Catecholamines*

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Macrophage migration inhibitory factor (MIF) is a relatively small, 12.5-kDa protein that is structurally related to some isomerases and for which multiple immune and catalytic roles have been proposed. MIF is widely expressed in tissues with particularly high levels in neural tissues. Here we show that MIF is able to catalyze the conversion of 3,4-dihydroxyphenylaminechrome and norepinephrinechrome, toxic quinone products of the neurotransmitter catecholamines 3,4-dihydroxyphenylamine and norepinephrine, to indolehydroxy derivatives that may serve as precursors to neuromelanin. This raises the possibility that MIF participates in a detoxification pathway for catecholamine products and could therefore have a protective role in neural tissues, which as in Parkinson's disease, may be subject to catecholamine-related cell death.

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pressed in BL21 (DE3) pLysS cells and induced with 0.4 mM IPTG for Madison, WI). Following the manufacturer’s protocol, protein was ex-
then subcloned into the pCRII vector (Invitrogen, Carlsbad, CA) and sequenced and sequenced. L-DOPA, DN, NE, and EP were purchased from Sigma; the “chrome” 
products was performed using HPLC under standard conditions (33). Briefly, substrates were dissolved in 0.1M phosphate buffer (pH 7.0) and eluted with a linear gradient from 0 to 90% acetonitrile in 5% acetic acid. The mass 
spectrometer was scanned from 600–1600 m/z with a spectrum re-
corded every 3 s.

Enzymatic Assays—Use of various substrates and identification of products was performed using HPLC under standard conditions (33). Briefly, isoaromatic elution with 0.15 x sodium borate buffer (pH 2.5), 25% methanol was used with a C18 column at a flow rate of 0.5 ml/min. Peaks were detected using UV absorbance (280 nm), and pmol were calculated based on the absorption of known standards. L-DOPA, L-
DOPA, DN, NE, and EP were purchased from Sigma; the “chrome” derivatives of those substrates were generated as described previously (34). Briefly, substrates were dissolved in 0.1 M phosphate buffer (pH 6.8) and cooled to 4 °C; they were mixed continuously for 3 min on ice. Enzymatic assays were performed with various 
substrates and purified MIF at 37 °C for 1 h; reactions were stopped by the addition of an equal volume of the acidic HPLC buffer, and 20 µl was injected on the column. DHI and DHICA used as standards were gener-
ous gifts of Prof. Shosuke Ito (Fujita Health Sciences University, Nagoya, Japan). Spontaneous oxidation of all substrates was determined in the presence of buffer without MIF and has been subtracted from the results.

RESULTS

MIF Expression in Brain—MIF is widely expressed in new-
born mouse and fetal human tissues without regard to their immune status (4, 35). To examine expression of MIF mRNA in the adult human brain, Northern blots for different regions of the brain were probed with human MIF cDNA (Fig. 2). High levels of MIF expression were noted in all regions of the adult human brain, including the substantia nigra.

Preparation and Purification of Recombinant MIF—Recom-
binit MIF was prepared to examine catalytic activity(s) to-
ward possible physiologically relevant substrates. Mouse MIF 
cDNA (4, 5) was amplified by RT-PCR of mouse lens total RNA and was cloned into the pET17b expression vector. Because of the high conservation of residues at the N and C termini of MIF, which may be involved in enzyme activity (12–15), no fusion peptides were included. High yields of MIF protein were obtained after induction by IPTG (Fig. 3A, inset).

Recombinant MIF was then isolated from these extracts by fast protein liquid chromatography. Four fractions containing MIF were obtained by gel filtration (Fig. 3A) and were analyzed by SDS-polyacrylamide gel electrophoresis (Fig. 3A, inset), by two-dimensional gel electrophoresis, and by Western immuno-
 blotting (data not shown). Fraction D contained a relatively low level of MIF (apparently in association with some nonprotein absorbance), whereas Fractions A–C contained comparable amounts of MIF. Multiple forms of MIF have been previously reported (36–38). Fractions A–C were subjected to mass spec-
troscopy (Fraction B is shown in Fig. 3B) and showed identity with the size expected for native mouse MIF (12,372 Da), with an additional minor component 131 Da greater in size, presumably due to failure to remove the N-terminal initiator methio-
nine. All fractions thus contain chemically similar MIF, and our preliminary results show that these fractions are in dy-
namic equilibrium involving at least monomer and trimer forms. Other studies have implicated trimeric MIF as the principal native form (12–14, 39) although other native forms of MIF may exist (32, 40, 41). Fractions A, B, and C were deter-
determined to be enzymatically active in preliminary experiments. In this study, we concentrated on the substrate specificity of Fraction B because ultracentrifugation confirmed that 95% of MIF in that fraction was present as a trimer, the rest being in monomeric form. Fraction B has also been tested for biological activity, including the macrophage migration inhibition capil-
lar assay, and was found to be active (11).
**DISCUSSION**

Neural tissue, particularly brain, maintains a relatively high level of MIF mRNA, and our laboratory and others (42–44) have shown that MIF has a steady level of expression in neuronal tissues. Because MIF seems to be a multifunctional protein, it could have more than one role there. As evidence accumulates that MIF belongs to an enzyme superfamily (12–19, 45), a catalytic role seems likely. Previously it was found that MIF and the related D-Dct are capable of catalyzing the conversion of D-DC to the indole-quinones DHI or DHICA (16, 17). Although the D-stereoisomer of DC is not known to occur naturally, it is chemically related to other physiologically relevant compounds, such as DN, NE, and EP and their chroman derivatives, which are also derived from tyrosine during catecholamine biosynthesis (Fig. 1). Several of these compounds have great significance for brain function. Most notably, DN and NE are neurotransmitters, and the loss of dopaminergic neurons is associated with Parkinson's disease, a progressive neurological degeneration (20, 21). DN itself has been shown to induce apoptosis in neural cells and thymocytes (22–25). Furthermore, oxidation of DN, NE, or EP leads to the formation of highly toxic quinones, including DNc, NEc, and EPC. These quinones are known to be precursors of neuromelanin, a pigment that accumulates in neurons and astrocytes, particularly in the substantia nigra (26, 27). As shown here, MIF can readily metabolize DNc to DHI, NEc to THI, a DHI-like indole-quinone, and, to a lesser extent, EPC to THMI. All these DHI-like indole-quinoines can undergo spontaneous oxidation and polymerization into melanins, although DNc seems to be the most relevant precursor of neuromelanin formation (26, 29). Thus MIF has the potential to contribute to the biosynthesis of neuromelanin. Various roles have been suggested for neuromelanin; however, it seems likely that it acts as a sink for toxic products of catecholamine synthesis, including DNc and NEc (26, 27, 31).

Melanin biosynthesis occurs within melanocytes in skin, hair, and eye and requires the enzymatic activity of tyrosinase and several tyrosinase-related proteins, known as Tyrp1 and Dct (Fig. 1). Interestingly, Dct is a melanocyte-specific enzyme considered to be a "rescue" enzyme essential for melanocyte survival (46, 47). Mutations in Dct that decrease catalytic function affect DHICA production and are generally quite toxic to melanocytes, whereas Dct mutations like Dct that increase catalytic activity might contribute to increased melanogenesis.

**TABLE I**

| Substrate → product | Activity | n  |
|---------------------|----------|----|
| D-DC → DHICA        | 123.0 ± 19.5 | 12 |
| L-DC → DHICA        | background | 3  |
| D-DOPA → DHI or DHICA | background | 3  |
| L-DOPA → DHI or DHICA | background | 3  |
| DN → DHI            | background | 3  |
| NE → DHI            | background | 3  |
| EP → DHI            | background | 3  |
| DNc → DHI           | 53.6 ± 4.7 | 4  |
| NEc → THI           | 26.9 ± 7.7 | 11 |
| EPC → THMI          | 8.0 ± 3.2  | 5  |

Enzyme Activity—As expected (16), recombinant MIF was positive for D-Dct activity (Table I), converting D-DC to DHICA (17). The catalytic activity of MIF was highly stereospecific, as previously reported; MIF was not active toward the L-isomer of DC, nor was it active toward the L- or D-forms of DOPA, the precursors of DC (Table I). Because the D-isomer of DC is not likely to be the physiological substrate of MIF (because the occurrence of D-DOPA or a DOPA racemase has never been reported), we next examined the potential catalytic function of MIF toward structurally related catecholamines, including DN, NE, and EP and their dihydroxyindole derivatives, DNc, NEc, and EPC (Table I). Although there was no activity of MIF toward DN, NE, or EP, it was quite active toward DNc and NEc and to a lesser extent EPC, converting them to their natural dihydroxyindole derivatives, DHI, 3,5,6-trihydroxyindole (THI), and 3,5,6-trihydroxyindole-1-methylindole (THMI), respectively.

Fig. 2. Northern blot analysis of MIF in adult human brain. MIF expression in various regions of the brain as noted was assessed by commercial Northern blots as detailed under "Experimental Procedures." Hybridization with a probe for β-actin is shown as a loading control.

Fig. 3. Synthesis and purification of recombinant mouse MIF. A, gel filtration of recombinant MIF. Four peaks containing immunoreactive MIF are labeled A, B, C, and D. Insert, SDS-polyacrylamide gel electrophoresis of Escherichia coli pLysS soluble extracts. Lane 1, bacteria containing the "empty" pET17b plasmid; lane 2, uninduced bacteria containing pET17-MIF; lane 3, bacteria with pET17-MIF induced by IPTG; lane A, MIF fraction A; lane B, MIF fraction B; lane C, MIF fraction C; lane D, MIF fraction D. Migrations of molecular mass standards are shown in kDa on the left; and the position of the 12.4-kDa MIF is indicated with an arrow on the right. B, mass spectrometry profile of purified recombinant MIF. Peaks correspond in size to MIF and to Met-MIF as shown.
melanocytes. Melanocytes typically express Dct before any of the other melanogenic enzymes, presumably to minimize such toxicity (48). Catecholamine biosynthesis occurs within neural cells and requires a set of distinct enzymes, including tyrosine hydroxylase, DOPA decarboxylase, DOPAmine β-hydroxylase, and phenylethanol amine-N-methyl transferase, for the production of DN, NE, and EP. Several mechanisms, enzymatic and nonenzymatic, have been proposed to result in the oxidation of these precursors to their cyclized chrome derivatives (27), and MIF should now be included in the neuromelanin biosynthetic pathway, as noted in Fig. 1. It may also have a similar protective function in catecholaminergic neurons in minimizing intracellular cytotoxicity, comparable with Dct function in melanocytes. To emphasize the importance of neuronal protective mechanisms against catecholamines, it is worth noting that other antioxidant activities contributing to prevent the formation of o-quinones derived from catecholamines have been reported that are based on the conjugation of these compounds to GSH catalyzed by class Mu glutathione transferase M2–2 (49). Interestingly, this GST isoenzyme and MIF share a proline residue surrounded by a number of aromatic residues in the N-terminal region, which forms a large hydrophobic pocket that has been proposed to be the active site interacting with the catecholamine substrate (50).

MIF is expressed throughout the adult brain and is therefore widely available to participate in the metabolism of oxidized catecholamines and related compounds. To what extent MIF participates directly in neuromelanin synthesis remains to be seen. However, MIF may have a direct role in helping to detoxify catecholamine-derived quinones, thereby contributing to protection of catecholaminergic neurons and surrounding cells. Indeed, it has been shown that mesencephalic glial cells produce soluble factors that protect dopaminergic neurons from the toxic effects of quinones (51). Conceivably MIF may participate in this detoxification process; for although DHI, the im-

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