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Chaperone-like activity of macrophage migration inhibitory factor

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Abstract

Macrophage migration inhibitory factor is a ubiquitous multifunctional cytokine having diverse immunological and neuroendocrine properties. Although this protein is known to be released into the circulation from the secretory granules of anterior pituitary or directly from immune cells as a consequence of stress, its participation in heat stress-induced aggregation of proteins has not yet been reported. We provide here the first evidence that the macrophage migration inhibitory factor possesses chaperone-like properties. It was shown to exist in the form of a mixture of low and high molecular weight oligomers. At heat stress temperatures the large oligomers dissociate into monomers that bind and stabilize thermally denatured malate dehydrogenase and glycogen phosphorylase *b* and thus prevent aggregation of the model proteins. Similar chaperone-like effects were also observed in the presence of partially purified brain extract containing besides the macrophage migration inhibitory factor a number of ubiquitous hydrophobic low molecular weight proteins identified by N-terminal microsequence analysis. Being highly stable and hydrophobic, the macrophage migration inhibitory factor in combination with other proteins of similar properties may comprise a family of constitutively expressed "small chaperones" that counteract the early onset of stress, around physiological conditions, when heat shock proteins are not abundant.

Keywords: Chaperone-like activity; Protein aggregation; Heat stress; Hydrophobic proteins; Macrophage migration inhibitory factor

1. Introduction

External stress (heat, oxidizing conditions, toxic compounds) or mutations may cause labile proteins to lose their distinct native conformations and seek alternatively stable aggregated forms. Molecular chaperones can protect the non-native proteins by binding hydrophobic unraveled or misfolded surfaces thereby preventing them from interaction with each other or with other proteins in non-productive or damaging ways. Non-native states of proteins occur on many other occasions, including co-translational

Abbreviations: MIF, macrophage migration inhibitory factor; MDH, malate dehydrogenase; Ph*b*, glycogen phosphorylase *b*; HSP, heat shock protein; SDS, sodium dodecyl sulfate; HEPES, *N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid]; ME, mercaptoethanol; TFA, trifluoroacetic acid; AcN, acetonitrile; HPP, *p*-hydroxyphenylpyruvate; PVDF, polyvinylidene difluoride; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; FPLC, fast performance liquid chromatography; HPLC, high-performance liquid chromatography

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folding of the synthesized polypeptide chains, protein translocation across membranes, the synthesis of individual proteins of supramolecular multisubunit assemblies, waiting for their destined binding partners (Beissinger & Buchner, 1998; Frydman, 2001; Gething, 1997).

Molecular chaperones comprise several structurally distinct protein families. The major classes of chaperones include heat shock proteins (HSPs), such as GroEL, DnaK, HSP90, and small HSPs (15–30 kDa). Some of them (HSP70, GroEL) facilitate refolding of the damaged proteins to the native state, whereas other chaperones (α-crystallins, small HSPs) suppress or prevent aggregation and stabilize proteins, but do not assist their refolding to the fully native conformation (Ben-Zvi, Chatellier, Fersht, & Goloubinoff, 1998; Buchner, 1996; Hartman, Surin, Dixon, Hoogenraad, & Hoj, 1993; Huang, Chen, Lui, & Zhou, 2002; MacRae, 2000).

Protein aggregates can be solubilized and their non-native conformers can be correctly refolded by a specific network of ATP-hydrolyzing chaperones and co-chaperones. They act sequentially through concerted systems of multichaperone network, often involving appropriate folding enzymes (peptidyl-prolyl *cis–trans* isomerase and protein disulfide isomerase) and even other molecular chaperones, chaperone-like proteins and peptides (Ben-Zvi & Goloubinoff, 2002; Ben-Zvi et al., 1998; Bhattacharyya, Santhoshkumar, & Sharma, 2003; Bischofberger, Han, Feifel, Schonfeld, & Christen, 2003; Huang et al., 2002; Manna et al., 2001).

Molecular chaperones are now known to eventually perform diverse functions assisting substrate proteins not only to fold properly but also to fulfil their destined roles in vivo: regulation of signal transduction pathways, involving enzymes and steroid receptors; translocation to a particular subcellular compartment; facilitated proteolysis of unstable proteins; assembly/disassembly of oligomeric or higher order structures (Frydman & Hohfeld, 1997; Gething, 1997; Hendrick & Hartl, 1993). Multiple types of chaperones in various compartments may have different functions. The efficiency of chaperone-mediated processes depends on the nature of the protein substrate, on environmental conditions, on the aggregate type, size, and solubility (Diamant, Ben-Zvi, Bukau, & Goloubinoff, 2000).

Though molecular chaperones known up today possess one common feature: they have hydrophobic domains exposed at the surface enabling them to recognize and to bind unfolded proteins, the structural features of a protein that make it a chaperone are far from being clear. Hence the keen interest to chaperones does not seem to relax, and the list of new chaperone-like proteins discovered is increasing quite rapidly.

In the course of the study of the primary structures and molecular mechanisms of action of heat-stable low molecular weight immunologically active compounds of the nervous system we have isolated from the soluble fraction of bovine brain a protein that was identified as macrophage migration inhibitory factor (MIF) (Gurvits et al., 2000).

MIF was originally defined as one of the first cytokines discovered and was first described as a factor that inhibited the random movement of macrophages in vitro (Bloom & Bennet, 1966). Recently it was "rediscovered" as an anterior pituitary gland hormone, then the presence of MIF has been demonstrated in many regions of the nervous system and other tissues (Galat, Riviere, & Bouet, 1993; Nishino et al., 1995). More recently MIF was characterized as an immunological mediator that counter-regulates glucocorticoid action and contributes to inflammatory response (Baugh & Donnely, 2003; Donnelly & Bucala, 1997). It is released from activated macrophages (Calandra, Bernhagen, Mitchell, & Bucala, 1994), T-cells (Bloom & Bennet, 1966) and eosinophils (Rossi et al., 1998) and by the anterior pituitary gland as part of a systemic stress response (Bernhagen et al., 1993; Donn & Ray, 2004; Nishino et al., 1995). Further studies demonstrated emerging evidence that MIF was produced at all levels of the hypothalamo-pituitary-adrenal axis, the system involved in adaptation to stress (Bacher et al., 1998). The localization of MIF in cells, which have contacts with cerebrospinal fluid and blood vessels suggests that MIF might play a role as a humoral factor. It may be useful in the study of the pathogenesis of a number of diseases such as neurodegenerative disorders, virus infection and tumor (Hudson et al., 1999; Nishio, Minami, Kato, Kaneda, & Nishihira, 1999). A number of catalytic functions of MIF (keto–enol tautomerase, thiol–protein oxidoreductase activities) have been also revealed (Kleemann et al., 1998; Rosengren et al., 1997).