CHARACTERIZATION OF THE MACROPHAGE MIGRATION INHIBITORY FACTOR-BINDING SITE OF SARCOLECTIN AND ITS RELATIONSHIP TO HUMAN SERUM ALBUMIN

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Summary: The sialic acid-binding protein sarcolectin from human placenta specifically interacts with the lymphokine macrophage migration inhibitory factor, enabling its convenient purification and histochemical localization. After cyanogen bromide-mediated cleavage of sarcolectin one polypeptide with an apparent molecular weight of approximately 15,000 exhibited binding capacity to the labelled lymphokine, as revealed by ligand blotting. The N-terminal sequence stretch of this peptide is identical to the respective sequence of human serum albumin, following the internal methionine residue in position 298. Cleavage at a methionine moiety in position 446 can explain the size of the 15 KDa product of chemical degradation. Close similarity of circular dichroism of sarcolectin and human serum albumin added further evidence to their structural similarity, calling for further studies to rigorously define their relationship.

Lectins participate in a wide variety of regulatory processes, also involving recognitive interactions in the immune system (1). The sialic acid-binding sarcolectin, present in various types of mammalian tissues, is known as α/β-interferon antagonist and growth modulator (2-5). Prompted by these obvious physiological activities, its major binding protein from human placenta has been purified by affinity chromatography (6). It is identical to a lymphokine, namely macrophage migration inhibitory factor, whose presently inferred activities include macrophage activation and potential potency as regulatory mediator in cell proliferation and differentiation or as key factor in the toxic response to endotoxaemia (6-10). In comparison to a lymphokine-specific antibody sarcolectin

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exhibits the same capacity to localize macrophage migration inhibitory factor in tissue sections, underscoring the specificity of the interaction (11). Notably, binding of sarcolectin, indicative for presence of the lymphokine, is correlated with good prognosis in lung cancer sections (12). As a step to define this interaction on the molecular level we herein report the characterization of the lymphokine-binding peptide of sarcolectin. Remarkably, the partial amino acid sequence of this peptide, obtained after cleavage with cyanogen bromide and identified after ligand blotting, is shown to be identical to a sequence stretch of human serum albumin between two methionine residues. Assessment of circular dichroism of human serum albumin and sarcolectin emphasizes the structural relationship between these two protein preparations.

MATERIALS AND METHODS

Purification of sarcolectin and generation of cleavage peptides. Sarcolectin was purified from salt extracts of human placenta by successive steps including treatment with pepsin (0.5 mg pepsin/ml extract at 37° C for 1 h), gel filtration on Sephacryl S-200 and fractionation on DEAE-cellulose, as described (4, 6). Further separation into subfractions was achieved by hydroxyapatite chromatography of sarcolectin or commercial human serum albumin, dissolved in 20 mM phosphate buffer (pH 6.8), by eluting the gel matrix at 40 mM, 70 mM, 110 mM and 650 mM phosphate, as described (13). Cleavage peptides were generated by treatment of sarcolectin (1 mg), dissolved in 0.5 ml of 70% formic acid, with cyanogen bromide (50 µl of solution containing 20 mg cyanogen bromide/ml CH_3CN). After 6 h at room temperature 5 ml H_2O were added, the solution was frozen and then lyophilized.

Ligand blotting. Macrophage migration inhibitory factor was purified by affinity chromatography on a sarcolectin-Sepharose 4B column (12 mg sarcolectin/ml) from salt extracts of human placenta, as described (6). Contaminating immunoglobulins were removed by subsequent passage over a protein A-Sepharose CL-4B column (6). The purified lymphokine was biotinylated using biotinyl-N-hydroxysuccinimide ester, as described (14). This probe was employed to visualize the peptides of sarcolectin with binding capacity. Cyanogen bromide-generated cleavage peptides were separated on a 15% polyacrylamide gel under denaturing and reductive conditions and were then electrophoretically transferred onto nitrocellulose (0.2 µm pore size). The blot was briefly washed with phosphate-buffered saline (pH 7.5) and residual binding sites were blocked with 0.5% Tween-20 in phosphate-buffered saline for 2 h at room temperature. Biotinylated macrophage migration inhibitory factor (20 µg/ml) was incubated in phosphate-buffered saline containing 0.05% Tween-20 for 12 h at 4° C with the blot. After extensive washing the blot was treated for 2 h at room temperature with a solution of 10 µg/ml streptavidin-peroxidase and after thorough washes with 4-chloro-1-naphthol/H_2O_2 as chromogenic substrates.

Amino acid sequence analysis. The cleavage peptides were separated by tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis on a 15% running gel (15). Following staining with Coomassie brilliant blue R-250 for 5 min the area of

the gel containing the lymphokine-binding peptide was cut out and electrophoretic separation was repeated after incubation of the gel slice in standard sample buffer. This purified cleavage peptide was electrophoretically transferred to a polyvinylidene difluoride membrane and N-terminal sequence analysis was carried out, as described (16).

Measurement of circular dichroism. Proteins were dissolved in phosphate-buffered saline (pH 7.5) and dialyzed for 24 h against this buffer. The dialyzed solutions were adjusted to a concentration of 0.2 mg/ml, based on the dye-binding assay, adapted for microtiter plates, with bovine serum albumin as standard (17). The circular dichroism of each solution was determined with a CD spectrometer (Model J-710/720; Japan Spectroscopic Co., Tokyo, Japan).

RESULTS

The specific binding of human macrophage migration inhibitory factor to sarcolectin prompted us to more closely define the respective domain(s) of sarcolectin that is responsible for this immunoregulatory interaction. Cyanogen bromide was employed to generate cleavage peptides of sarcolectin (Fig. 1a).

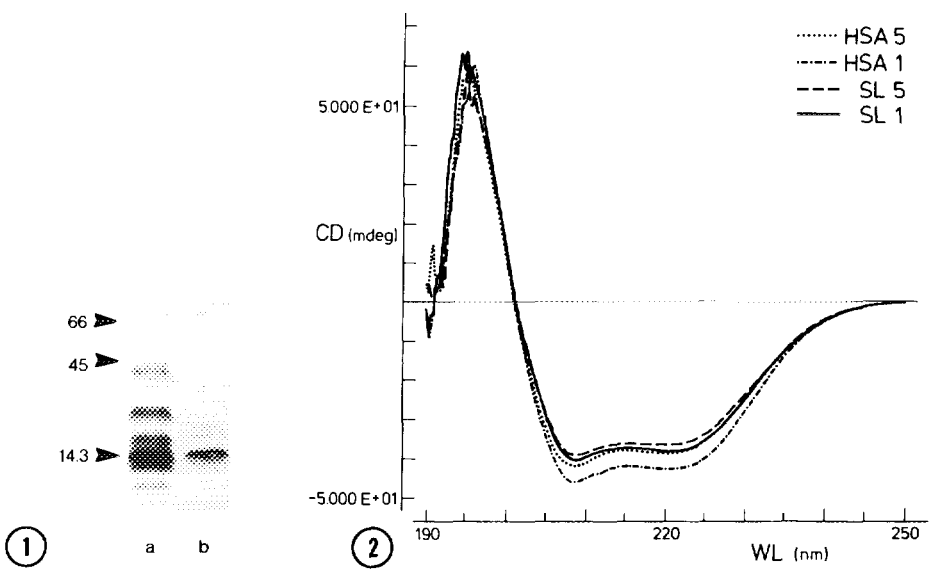

Fig. 1. Separation by SDS gel electrophoresis and visualization by silver staining of CNBr-generated cleavage peptides of sarcolectin (a) and visualization of the macrophage migration inhibitory factor-binding peptide after electrophoretic transfer to nitrocellulose, ligand probing with biotinylated lymphokine and color development with streptavidin-peroxidase and the substrates 4-chloro-1-naphthol/H_2O_2 (b). Positions of markers for molecular weight designation are indicated by arrowheads.

Fig. 2. Circular dichroism spectra of fractions of human serum albumin (HSA) and sarcolectin (SL), obtained after hydroxyapatite chromatography in the first and fifth step of subfractionation (fractions 1 and 5).

Among the ensuing products of site-specific chemical degradation of sarcolectin one polypeptide of an apparent molecular weight of approximately 15,000 consistently showed strong reactivity to the lymphokine in a ligand blotting assay performed with biotinylated macrophage migration inhibitory factor (Fig. 1b). This cleavage peptide was subjected to successive cycles of Edman degradation to determine its N-terminal sequence, resulting in unequivocal identification of the following amino acid stretch: Pro-Ala-Asp-Leu-Pro-Ser-Leu-Ala-Ala-Asp-Phe-Val-Glu. This sequence was found to be identical to a part of human serum albumin, following an internal methionine residue in position 298, which is located outside of any loop in the structure of albumin. Close examination of this observation is thus warranted. To explain the apparent molecular weight of the detected peptide, intraloop cleavage at the methionine moiety in position 446 of the sequence of albumin can be proposed. Thus, the sequence information suggests that sarcolectin is at least in part closely related to human serum albumin, assigning the lymphokine-binding domain to an internal sequence stretch between two methionine residues.

As a means to compare further structural aspects of the two proteins of human origin, namely sarcolectin and serum albumin, determination of their circular dichroism in the UV range was performed. Notably, no significant difference could be detected between the two protein preparations or their subfractions after hydroxyapatite chromatography (Fig. 2).

DISCUSSION

Enhancement of macrophage activity as well as participation in effector pathways that lead to fatal septic shock emphasize the importance of macrophage migration inhibitory factor in immunoregulation (9, 10). Similar to other modulatory proteins and the regulation of their activities by binding to soluble factors the specific interaction between this lymphokine and sarcolectin deserves attention beyond its application for purification and histochemical localization of the lymphokine (6, 11, 12). To improve the level of understanding of this interaction mapping of the binding site on sarcolectin is warranted. Generation of cleavage peptides of sarcolectin and biotinylation of the lymphokine, not impairing its binding capacity, has enabled to visualize a sarcolectin-derived peptide that bound the probe in ligand blotting. This polypeptide comprises less than a quarter of the molecular weight of native sarcolectin. Remarkably, its N-terminal sequence is identical to a stretch of amino acids in human serum albumin, starting at the methionine residue in position 298 (18, 19). The complete length of this peptide, measured by SDS-polyacrylamide gel electrophoresis, is compatible with the size of a fragment of albumin, obtained by further cleavage at

the methionine residue in the intraloop position 446. Additionally, it should be noted that the molecular weight values of several other fragments of sarcolectin are in agreement with results from measurements with human serum albumin under similar conditions, pointing to a comparable distribution of the methionine residues in the two protein preparations (20-22). The study of the circular dichroism corroborates the assumption of a close relationship between albumin and sarcolectin.

In human serum albumin the fragment from positions 298-445 comprises a part of the intradomain connection between the subdomains IIA-IIB, the subdomain IIB, the connection to domain III and part of subdomain IIIA, which ends at the glutamic acid at position 492 (23). Principal ligand binding sites have been assigned to the subdomains IIA and IIIA (18, 23). Interaction with another protein, the streptococcal protein G, has been found for the cleavage peptide, consisting of the amino acids between position 330-446, a 5.5 kDa pepsin fragment that starts at position 424 being a strong inhibitor (22). This example suggests potential importance of this part of the sequence of albumin for recognitive interactions and also indicates that respective peptide pieces can effectively interfere with the specific binding of albumin to another protein. Further work will now focus on the delineation of the inferred relationship between albumin and sarcolectin and on the physiological significance of binding of macrophage migration inhibitory factor to sarcolectin that may be reduced by addition of a defined fragment of sarcolectin.

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