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Sequence similarity between protein B and human apolipoprotein A-IV

Jörg Rühlmann, Volker Kruft*, Brigitte Wittmann-Liebold* and Franz-J. Fehrenbach

Robert Koch-Institut des Bundesgesundheitsamtes, Abt. Mikrobiologie, Nordufer 20, D-1000 Berlin 65 and **Max-Planck-Institut für molekulare Genetik, Abteilung Wittmann, Ihnestr. 63–73, D-1000 Berlin 33, Germany*

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Sequence comparison of protein B (CAMP-factor) with human apolipoprotein A-IV (apo A-IV) revealed 32% similarity between the N-terminal part of protein B and a part of the putative lipid-binding domain of apo A-IV. The significance of this similarity is discussed with respect to the structure/function relationship of protein B.

Protein B; CAMP-factor; Apolipoprotein A-IV; Sequence similarity; Lipid-binding domain

1. INTRODUCTION

Protein B (CAMP-factor) was first described in 1944 by Christie et al. [1] as an extracellular product of group B streptococci (GBS, *Streptococcus agalactiae*), which causes hemolysis of sphingomyelinase-treated sheep erythrocytes. Protein B was purified from the culture supernatant of GBS to homogeneity [2] and the amino acid sequence has been determined recently [3]. In addition to the cohemolytic activity protein B is a Fc-binding protein [4] and hence exhibits properties similar to those of protein A of *Staphylococcus aureus* and the Fc-binding proteins of a variety of streptococci [5].

The mechanism by which protein B lyses susceptible erythrocytes or target liposomes was studied in detail by a number of authors [6–9]. Sterzik et al. [9], using artificial membranes, demonstrated that binding of protein B to liposomes is an undirected receptor independent process probably mediated by the amphiphilic nature of the polypeptide. Studies with purified CNBr-fragments of protein B suggested that protein B may consist of two different functional domains responsible for the interaction with lipids and the binding to the Fc-part of immunoglobulins [9,10]. Additionally, it was found that only the C-terminal part of protein B shows sequence similarity with a segment of the Fc-binding region in protein A [3].

This report describes a sequence similarity between the N-terminal part of protein B and human apolipoprotein A-IV (apo A-IV) [11] and investigates its possible significance with respect to the structure/function relationship of protein B.

Correspondence address: J. Rühlmann, Robert Koch-Institut des Bundesgesundheitsamtes, Abt. Mikrobiologie, Nordufer 20, D-1000 Berlin 65, Germany

2. MATERIALS AND METHODS

2.1. *Analysis of the sequence similarity*

The apo A-IV sequence (position 144–256) [11] and the protein B sequence (position 1–118) [3] were aligned by the algorithm of Needleman and Wunsch [12] with the UWCG program GAP. A gap weight of 5 and a gap length weight of 0.3 were used. Additional analysis was performed with the program ALIGN [13]. In this case the Unitary Matrix was used and the break penalty was set to 2. All computer analyses were performed on a Vax/VMS system.

2.2. *Secondary structure predictions*

The secondary structure of protein B was predicted according to the methods of Chou and Fasman [14], Burgess et al. [15] and Nagano [16] as described [17,18]; the calculation of the predictive algorithms was performed on a Vax/VMS system using the program PREDICT NORM (Dzionara, M. and Wittmann-Liebold, B., unpublished).

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3. RESULTS AND DISCUSSION

Comparison of the amino acid sequence of protein B [3] with sequences from the NBRF database (release 13.0) revealed a similarity between partial sequences of protein B and human apo A-IV [11]. In fig.1 the corresponding segments of both sequences were aligned using the UWCG program GAP. The alignment was optimized by the introduction of three gaps. There are 25 identities out of 112 possible matches within the region of maximal sequence similarity extending over 118 residues (position 1–118: 22% identities). If conservative replacements are taken into account the similarity increases to 32%. A similar result was obtained when the sequences are aligned with the program ALIGN using the Unitary Matrix (not shown).

The similarity between apo A-IV and protein B is restricted to the N-terminal part of protein B (position 1–118). Interestingly, the 9 kDa CNBr-fragment (position 22–107) which represents most of this N-terminal part of protein B has been shown to bind to lipid suspensions of cholesterol and sphingomyelin, whereas the 13.5 kDa CNBr-fragment (position 108–226) corresponding to the C-terminal part of protein B did not bind [9].

The apo A-IV sequence shown in fig.1 belongs to a segment of mature apo A-IV which is composed of several 22-amino-acid-long repeats. These repeats have the potential to form amphiphilic helices and it has been suggested that they represent the lipid binding structures of apo A-IV [19].

We think that the weak but still significant similarity between the N-terminal part of protein B and the putative lipid binding domain of apo A-IV may reflect a common function of both sequences namely to interact with lipids.

Because amphiphilic helical structures seem to be responsible for the lipid-binding properties of various proteins and peptides which interact with lipids or membrane surfaces [20–23], we examined the protein B sequence for segments with the potential to form amphiphilic helices. Secondary structure prediction of the N-terminal part of protein B according to Chou and Fasman [14] reveals the existence of four α-helical segments (fig.1).

In addition to the Chou and Fasman algorithm the prediction methods of Burgess et al. [15] and Nagano [16] were applied for the prediction of α-helical segments (table 1). These segments were ex-

Table 1

Comparison of the position of the predicted α-helices within protein B (position 1–118) with the amphiphilic helices from fig.2

Position of the predicted helix according to	Helix			
	1	2	3	4
Chou and Fasman [14]	17–25	47–59	83–89	97–107
Burgess et al. [15]	19–28	51–57	80–88	–
Nagano [16]	17–25	48–52 + 54–63	79–89	93–103
Amphiphilic helix (fig.2)	17–28	48–58	79–89	92–103

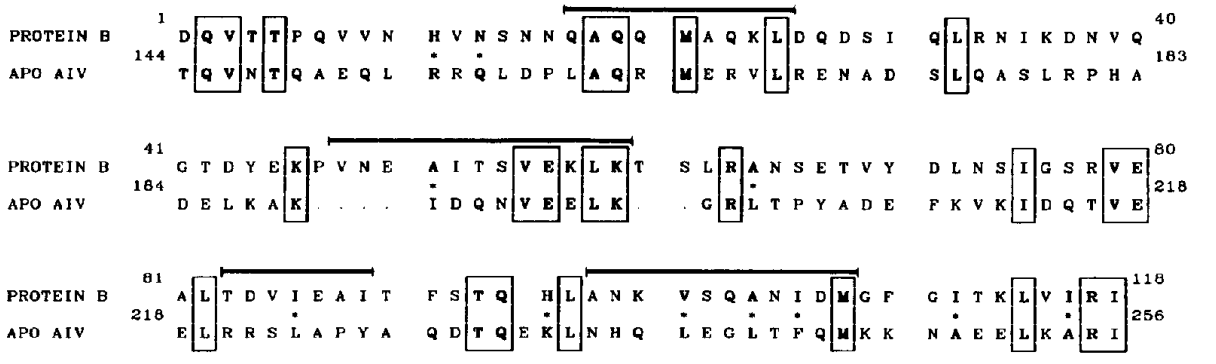

Fig.1. Comparison of partial sequences of protein B and human apolipoprotein A-IV. Identical residues in both sequences are boxed; conservative replacements are indicated by asterisks (*). Three gaps have been introduced for better alignment. Horizontal bars indicate α-helical segments according to the Chou and Fasman prediction [14].

amined for amphiphilic characteristics by the helical wheel projection as described originally by Schiffer and Edmundson [24] (fig.2). The axial projection shows the amphiphilic nature of all four helices: hydrophilic and hydrophobic residues are segregated on opposite sides of the helix. The hydrophobic domain occupies almost 180° of the cylindrical surface. Although the length of the potential amphiphilic helices in fig.2 (11–12 amino acids) is shorter than that of amphiphilic helices of other proteins and peptides (20–23), it corresponds to the average length of an α-helical structure in globular proteins of 11 residues or three helical turns [25]. Interestingly, identical and homologous amino acids present in apo A-IV and protein B were found to be predominantly clustered within and around the predicted amphiphilic helices.

In addition to the Edmundson wheel diagram the Kyte-Doolittle plot further indicates that these helices have the potential to form amphiphilic structures: plotting the hydrophobic index of each residue in the sequence as a function of its residue number results in a pattern that alternates regularly between hydrophobicity and hydrophilicity (not shown). A similar alternating pattern was obtained for the potential amphiphilic helices of apo A-IV [19]. Examination of the C-terminal part of protein B (position 121–226) reveals that it contains no potential amphiphilic helical structures.

The sequence similarity described in this paper resembles the finding of Lambotte et al. [26]. They reported on a sequence similarity between diphtheria toxin and human apolipoprotein A-I. The 77-residue string of diphtheria toxin, exhibiting 25% identical amino acids with a segment of apolipoprotein A-I, is located in fragment B, which is known as the membrane binding unit of diphtheria toxin. Lambotte et al. [26] suggested that the two amphiphilic α-helices within this 77-residue string may interact with the surface of phospholipid bilayers ('surface lipid associating domain').

Our structural analysis of the protein B sequence presented in this paper, supported by the experimental data of Sterzik et al. [9], suggests that the structural similarity of partial sequences from protein B and apo A-IV reflects the common property of lipid binding. In conclusion, the N-terminal segment of protein B (position 1–118) seems to represent the membrane binding domain of protein B and the potential amphiphilic helices may be involved in protein/lipid interaction. In contrast, the C-terminal part of protein B which exhibits similarity to a segment of the Fc-binding region of protein A, seems to represent the Fc-binding domain. The detailed structural analysis of the protein B sequence presented in this report supports the hypothesis that two different functional domains, responsible for lipid-binding and Fc-binding, exist in protein B (fig.3).

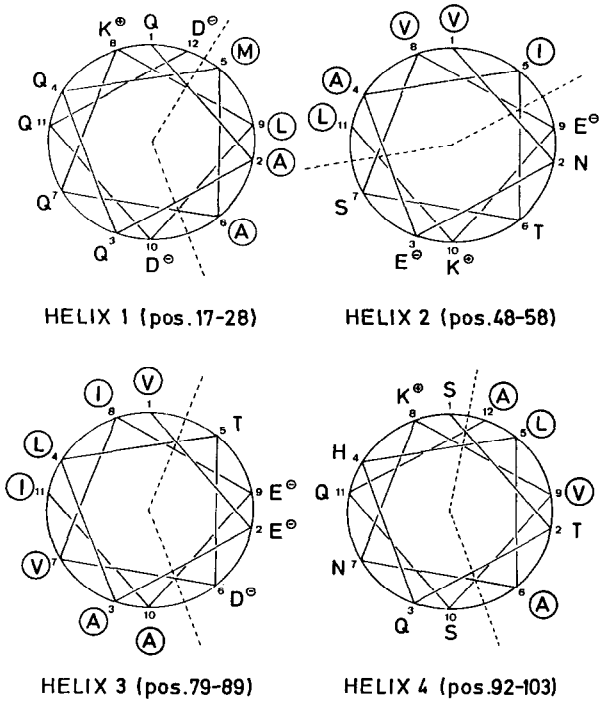

Fig.2. Helical wheel-plots of the potential amphiphilic helices of protein B. Hydrophobic residues are shown in circles. The dashed line separates hydrophobic and hydrophilic residues.

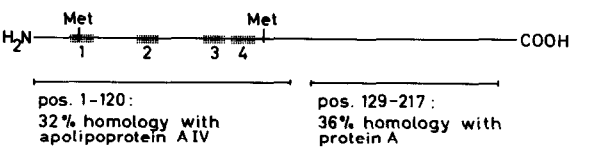

Fig.3. Putative localization of the two functional domains within the protein B sequence. The degree of homology implies identities and conservative replacements. The position of the amphiphilic helices of fig.2 is indicated by hatched rectangles.

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