THE NH_2-TERMINAL AMINO ACID SEQUENCE OF CELLULAR RETINOIC-ACID BINDING PROTEIN FROM RAT TESTIS

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1. Introduction

Several intracellular water-soluble proteins with high affinity for vitamin A compounds have been identified [1–5]. Two of these proteins, the cellular retinol-binding protein (CRBP) and the cellular retinoic acid-binding protein (CRABP) have been proposed to mediate the physiological action of vitamin A in some tissues [6,7]. CRBP has a widespread tissue distribution and occurs in epithelial tissues as well as in the liver [8]. CRABP is present in the adult only in a few tissues, among them the gonads [8]. Both proteins have been purified to homogeneity from several sources [9–13].

The tentative amino acid sequence of rat liver CRBP has been determined [14] and it displays significant homology with the myelin protein P2 [15]. Work in progress is aimed at elucidating the three-dimensional structure of rat liver CRBP by X-ray crystallography [16].

Rat CRABP has not yet been investigated in terms of its primary structure. However, we report here the NH_2-terminal amino acid sequence of rat testis CRABP. The data demonstrate that the 2 retinoid-binding proteins are related to each other as well as to the myelin protein P2.

2. Materials and methods

2.1. *Isolation of CRABP*

CRABP was isolated from rat testis essentially as in [10]. Upon high-resolution SDS–polyacrylamide gel electrophoresis the purified protein separated into 2 very closely migrating bands. However, this heterogeneity, whether of genetic origin or due to post-translational modification is not derived from the NH_2-terminal region. This can be inferred from the observation that the highly purified CRABP gave rise to a single NH_2-terminal amino acid sequence with the expected yield of phenylthiohydantoin-amino acids in the degradation steps.

2.2. *Protein sequence determination*

CRABP, extensively reduced and alkylated, was exhaustively dialysed against water, and 36 nmol were subjected to automatic NH_2-terminal amino acid sequencing using a Beckman 890C sequencer. The details of the procedure have been given [17]. The phenylthiohydantoin-amino acids were analysed by reversed-phase, high-pressure liquid chromatography [18]. The repetitive yield, calculated from the yields of the leucine residues in positions 18,19,22 and 28 was found to be ~98%. The same calculation based on the alanine residues in positions 4,21,26 and 32 gave a repetitive yield of 96%.

3. Results and discussion

Highly purified rat testis CRABP was subjected to NH_2-terminal amino acid sequence analysis. The amino acid sequence obtained allowed unambiguous identification of 32 residues (fig.1). The NH_2-terminal amino acid could not be identified due to the high background in this step.

The comparison of the CRABP sequence with the amino acid sequences of rat liver CRBP in [14] and the bovine myelin protein P2 in [15], reveals that to maximize homologies the CRABP sequence has to start with a gap, i.e., CRABP appears to be one amino acid shorter than CRBP and P2 in the NH_2-terminal

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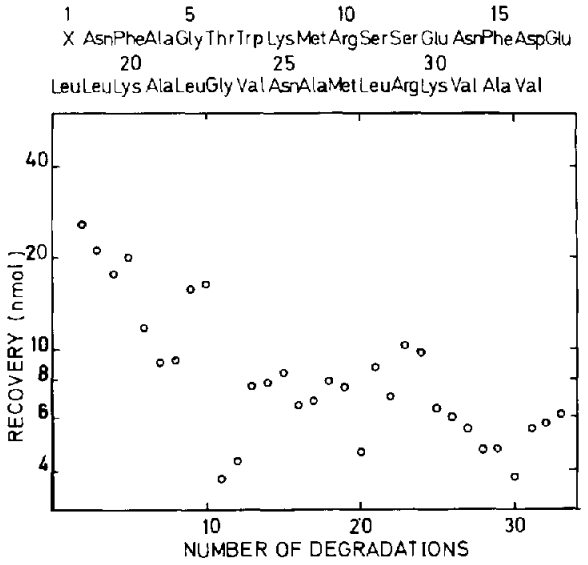

Fig.1. The NH_2-terminal amino acid sequence of 36 nmol rat CRABP. Phenylthiohydantoin-amino acids were identified and quantitated by reversed-phase, high-pressure liquid chromatography.

region. Of the 32 positions available for comparison, CRBP and CRABP have identical amino acid residues in 19. Moreover, of the 13 differences 7 may be accounted for by single base substitutions. Overall, CRBP and CRABP display such a high degree of homology that it is surprising that, antigenically, they appear to be totally different [19]. However, in the remainder of the primary structure of the 2 proteins differences may be more pronounced.

CRABP also shares amino acid sequence homology with P2. In 19 out of the 32 positions available for comparison the 2 proteins are identical. Consequently, CRABP seems to be as similar to P2 as it is to CRBP. These homologies strongly suggest that CRABP,

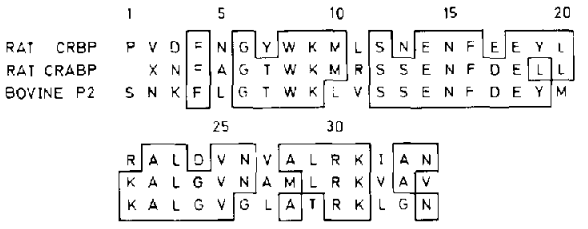

Fig.2. Comparison of rat CRBP, rat CRABP and bovine P2. Identical residues are enclosed in boxes. The numbering is based on the sequences for CRBP and P2 [14].

CRBP and P2 have an interelated evolution. Since the 3 proteins do not display any significant homology to other proteins, whose primary structures have been determined, it appears reasonable to conclude that CRABP, CRBP and P2 represent the first 3 members of a new protein superfamily.

The high degree of specificity of CRBP and CRABP for their ligands retinol and retinoic acid, respectively, and the observed homologies of these proteins with the myelin protein P2 may suggest that the latter also interacts with some kind of polyisoprenoid derivative. However, such a ligand has not yet been identified.

After completion of this study, the NH_2-terminal amino acid sequence of CRBP and CRABP isolated from bovine retina was reported [20]. When the amino acid sequences of CRBP, CRABP and the myelin protein P2 obtained from different species [15,21], are compared to each other, it is obvious that all proteins show remarkably conserved structures. Rat and bovine CRBP display identity in 27 out of the 30 positions available for comparison (90%) and rat and bovine CRABP are completely identical in the corresponding region. The myelin protein P2, isolated from bovine [15] and rabbit [21] nerve tissues are identical apart from 4 positions out of the 64 first residues (94% homology).

The highly conserved structures of the proteins belonging to this new protein family may have a functional significance. Thus, it would not be surprising to find that the proteins interact with highly conserved structures in the cell. The functions of these proteins are largely unknown although some evidence suggests that CRBP may participate in the transport of retinol into the nucleus [22,23].

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