Identification of the stable free radical tyrosine residue in ribonucleotide reductase

A sequence comparison

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Received 11 February 1985

The small subunit of ribonucleoside diphosphate reductase contains a unique tyrosine radical and a binuclear iron center. An alignment of different primary structures of the small subunit in *Escherichia coli*, the marine mollusc *Spisula solidissima*, Epstein Barr and Herpes simplex viruses shows that regions comprising residues 115–122, 204–212 and 234–241 (in *E.coli* numbering) are strikingly similar and are likely to be recognized as functionally important. Two of 16 tyrosine residues and 2 of 8 histidine residues are conserved. We propose that Tyr-122 is responsible for radical stabilization and that His-118 and His-241 together with Glu-115 and Asp-237 or Glu-238 are ligands of the iron center.

Ribonucleotide reductase *Secondary structure prediction* *Sequence comparison* *Tyrosine radical*

1. INTRODUCTION

Ribonucleotide reductase is an essential constituent of all living cells [1]. It provides precursors for the DNA synthesis by catalyzing the reduction of all 4 ribonucleotides to their corresponding deoxyribonucleotides. The nucleotide sequence of the ribonucleotide reductase operon in *Escherichia coli* was recently determined [2]. The enzyme consists of two non-identical subunits, proteins B1 and B2, the primary structures of which were deduced from the nucleotide sequence.

Recently, nucleotide sequences encoding the reductases of other genera have been reported. A 140-kDa protein and a 38-kDa protein in the HSV2 genome have been assigned to ribonucleotide reductase subunits based on genetic, immunological and enzymatic data ([3–7], Ingemarson et al., in preparation). Because of similarities to the HSV2 coding sequences a 93-kDa and a 34-kDa protein from an equivalent area of the EBV genome are also believed to code for ribonucleotide reductase subunits [8]. In clams and sea urchins the most abundant mRNA stored in unfertilized eggs, which is preferentially translated immediately after fertilization codes for a B2 equivalent protein of about 42 kDa (Standart et al., in preparation). Recent data clearly show that the 42-kDa product in urchins is involved in reduction of ribonucleotides in this species (unpublished).

The most interesting feature of the small subunit of ribonucleotide reductase is that it contains a stable free radical localized to a tyrosine residue [9]. This fully conserved tyrosine radical is essential for the activity of the enzyme. The tyrosine radical is located close to and stabilized by a binuclear iron center which has very similar spectral properties to hemerythrins.

Here, all available protein sequences (derived

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00145793/85/$3.30

from DNA sequences) have been aligned in order to extract information about possible iron ligands and tyrosine residues.

2. METHODS

The amino acid sequences were searched pairwise for highly homologous regions using several different computer programs. From these regions the alignments were extended manually trying to keep them as similar as possible. Gaps were avoided as much as possible.

Secondary structure predictions were calculated with the program of Garnier et al. [10]. A joint prediction was summed for all the common regions of the alignment.

Model-building studies of iron centers were performed on a Vector General VG 3404 linked to a Digital VAX 11/750 using the FRODO program [11,12].

3. RESULTS AND DISCUSSION

Protein B2 of *E. coli* has a low degree of sequence homology to the corresponding eucaryotic and virus coded B2 equivalents. All 4 sequences can be aligned because of a few specific regions which are strikingly similar in all proteins. The sequence alignment shown in fig.1 results in 23% homology between the *E. coli* and clam proteins and 16–17% homology between the *E. coli* and the virus proteins (HSV2 and EBV). In this set of 4 different B2 proteins only the EBV and HSV2 sequences show a more pronounced homology of 36% [8]. The similarities are primarily manifested in the regions 115–122, 204–212 and 234–241 (numbering refer to B2 of *E. coli*). The large differences in the N-terminal parts are compatible with comparisons of the 38-kDa sequences in type 1 [13] and 2 HSV [14]. These closely related sequences are identical except for the 30 N-terminal

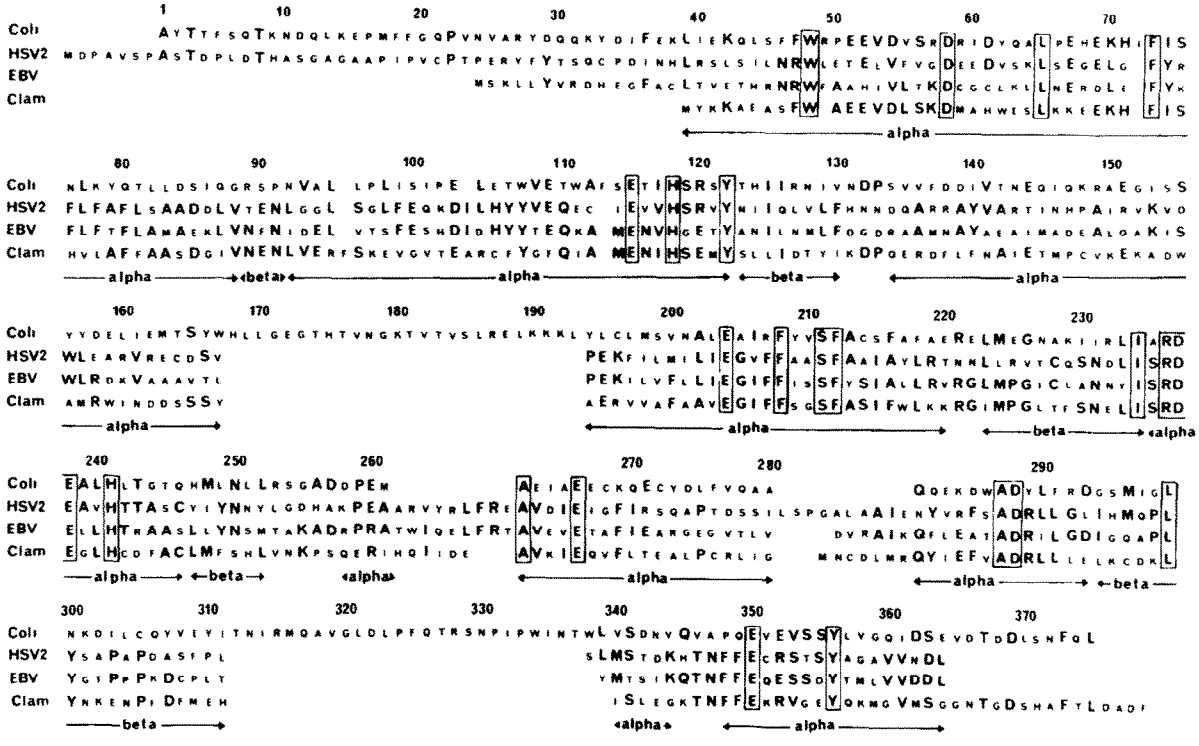

Fig.1. Amino acid sequence alignment of *E. coli* protein B2 [2], herpes simplex virus 38-kDa protein [3], Epstein Barr virus 34-kDa protein [8] and *Spisula solidissima* 42-kDa protein (Standart et al., in preparation). Structurally equivalent residues are in big capitals. Invariant residues of all 4 molecules are within boxes. The numbering refers to the *E. coli* sequence. Summed secondary predictions are indicated in the bottom line.

residues, which differ by approx. 50% [15]. Between the homologous regions, the proteins have variable chain lengths and generally the *E. coli* B2 protein is considerably longer than the others. Such differences may correspond to loop regions and be important for specific interaction with corresponding B1 equivalents. Such interactions are known to be very strict in that *E. coli* and bacteriophage T4 subunits cannot cross-react [6], neither can mammalian and HSV induced subunits [17,18].

The distant relationship utilized here has the consequence that few residues are common to all proteins, but has the advantage that residues important to the function show up. Here we wish to extract information about residues involved in the prosthetic group of B2, the tyrosine radical and liganding residues of the iron center. Only two of the 16 tyrosines, Tyr-122 and Tyr-356, in B2 are conserved in all sequences (fig.1) and are candidates to carry the free radical. Tyr-356 can be excluded because proteolytic cleavage of 29 residues at the carboxyl end of the B2 subunit results in a protein which still has an intact iron center and the tyrosine radical (Sjöberg et al., in preparation). We therefore propose that Tyr-122 is the free radical residue.

Protein B2 was recently shown to contain a μ-oxo-bridged binuclear iron center of the same type as is found in met-forms of hemerythrin [19]. In hemerythrin the iron center is liganded by one aspartic, one glutamic and 5 histidine residues [20]. Spectral properties of hemerythrin and B2 were found to be similar to an iron-containing model compound where the iron atoms are bridged by histidines and carboxylates [21]. It is thus very plausible that protein B2 will have a similar arrangement around its iron center. Of the 8 histidines in the *E. coli* B2 sequence two coincide in all 4 proteins (fig.1) and are thus very probable iron ligands. Both His-118 and His-241 lie in regions with high homology. The acidic residues are more frequently conserved and 5 of the 31 glutamic acids and 3 of the 25 aspartic acids are common to all proteins.

The predicted structure for the proteins suggests that both conserved histidines are located in helices (fig.1). Close to His-118 is found the conserved Glu-115 about one turn earlier in the helix and Tyr-122 about one turn later at the end of the helix. These residues thus come close in space and Glu-115 and His-118 can ligate the iron atom with the Tyr side chain in proximity to the iron center. Similarly, in the region around His-241 both Asp-237 and Glu-238 are approximately one turn earlier in the helix and one of these may also be an iron ligand.

The predominantly α-helical prediction for residues 39–122 (fig.1) fits with secondary elements observed in the known 3-dimensional structure of hemerythrin [20,22]. In fact, Glu-115 His-118 Tyr-122 of B2 align well in primary and secondary structure with the iron ligands His-101 and Asp-106 and the adjacent Tyr-109 in hemerythrin. However, the residues Glu, His and Tyr come in different order in B2 as compared to hemerythrin, and there is no further sequence homology between the N-terminal part of B2 and hemerythrin. Thus, we cannot find any evolutionary relationship between these two proteins. The limited number of conserved histidine residues in the B2 proteins support the suggestion that each polypeptide chain of the B2 protein contributes only half of the iron ligands and that the dimeric protein forms the binuclear iron center [9,19].

The comparison of distantly related B2 subunits of ribonucleotide reductase thus propose that Tyr-122, His-118, His-241, Glu-115 and Asp-237 or Glu-238 are functionally important residues. By the use of site-directed mutagenesis, it should be possible to directly determine whether these residues are required for enzymatic function. Alternatively, analysis of randomly generated mutants [23] defective in B2 activity should aid in identifying these residues.

ACKNOWLEDGEMENTS

This investigation was supported by grants from the Swedish Medical Research Council (project 3X-6801), the Swedish Natural Science Research Council (project K2767), Magn. Bergvall Foundation, the US Public Health Grant (GM 20884) and US National Science Foundation (project PCM 82-16917).

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