Biochimica et Biophysica Acta, 1058 (1991) 38–41

ADONIS 000527289100144I

BBABIO 43401

Bacterial 4-α-helical bundle cytochromes

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(Received 5 February 1991)

Key words: Cytochrome *c'*; Bacterioferritin; Hemoferritin; Ferritin; 4-α-helical

The biological functions of cytochrome *c'* and bacterioferritin, both haemoproteins with a common 4-α-helical bundle structure, are discussed and an example given of one of Kamen's laws, namely: comparative studies of prokaryotic cytochromes and their eukaryotic counterparts are useful. In the present case, the comparison is between bacterioferritin and its animal counterpart, haemoferritin.

TABLE I

4-α-helical bundle cytochromes

Protein [a]	Size [b]	Axial ligand	Refs.
Class IIa cytochrome *c* (cytochrome *c'*)	≈125 aa/subunit monomer and dimers	mono-His	5 and refs. therein
Class IIb cytochromes *c* (cytochrome *c*-556)	≈125 aa monomers	His-Met	5 and refs. therein
E. coli cytochrome *b*-562	106 aa monomer	His-Met	20,21
Cytochrome *b*-557 (bacterioferritin)	≈160 aa/subunit "24mer"	Met-Met	22,23

[a] The class II cytochromes *c* occur in a wide range of bacteria including aerobic, facultative anaerobic and photosynthetic organisms [3,4,14]. Bacterioferritin is also widespread having been isolated from *Azotobacter vinelandii* [15], *E. coli* [16], *Pseudomonas aeruginosa* [17] and photosynthetic organisms [18,19].

[b] aa = amino acids.

Introduction

Definition of the relationships between the structures of cytochromes, their chemical properties and their biological functions has occupied many workers over the past 60 years and yet, despite their efforts, which have made some of the cytochromes amongst the best characterised of all proteins, it is still not clear how any of the electron transfer cytochromes operate.

Kamen [1] advocated what has turned out to be one of the most valuable approaches for the characterisation of the functions of cytochromes: namely, comparative studies of proteins from a wide range of prokaryotic sources in addition to studies of eukaryotic proteins. This approach led early on in the development of the field to the three-dimensional structure of *Rhodospirillum rubrum* cytochrome c_2 [2] which has had a profound impact on the formulation of mechanistic hypotheses [2–5]. Thermodynamic and kinetic studies have also benefited from this approach and, again, good examples are provided by class I cytochromes *c* [6,7]. Partly through the efforts of Kamen and his co-workers, the class I cytochrome *c* family, which includes eukaryotic cytochrome *c* and bacterial cytochrome c_2, has come to be recognised as a major grouping [3–5,8–10]. Another major cytochrome structural grouping covering both eukaryotes and prokaryotes is the cytochrome b_5 superfamily [11]. In both of these families the cytochromes have common units of secondary and ter-

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tiary structure as well as common haem coordination centres. It is this latter feature that is the basis of the most commonly employed cytochrome classification scheme (see Ref. 4 and references therein).

In the past 10 years it has become apparent that a group of cytochromes with very different optical spectra, and hence different haem coordination centres, has a common three-dimensional structure. This is the arrangement of four α-helices in a left twisted antiparallel bundle [12,13]. Two members of this group of cytochromes (Table I) are the subject of the present paper. Appropriately, as with many bacterial cytochromes, Kamen was amongst the first workers to isolate and study these proteins, as will be apparent from the reference list.

Cytochromes *c′*

Cytochrome *c′* was first isolated by Vernon and Kamen [24]. Unlike most *c*-type cytochromes, its iron is only five-coordinate [25,26], and thus its ligand field is weak with a corresponding high-spin, or quantum-mechanically admixed high-spin/intermediate-spin spin-state [27]. A variety of investigations seeking to identify cytochrome *c′* in situ or in chromatophore preparations have been reported (see Refs. 4, 5, 14 and references therein). The majority of these failed to identify clearly cytochrome *c′*, raising the question of whether the protein was modified on isolation [25]. For example, despite having distinctive EPR spectra [27] signals of ferricytochrome *c′* have not previously been observed clearly in EPR spectra of intact cells of *R. rubrum* or *Rhodobacter capsulatus* [28,29]. This led Monkara et al. [30] to reinvestigate this topic. We used EPR to study isolated ferricytochrome *c′* and whole cells of *Rb. capsulatus* grown photosynthetically. Only after the addition of oxidants did signals clearly due to ferricytochrome *c′* appear in the whole cell spectra. Thus, the unusual spin-state of cytochrome *c′* does not result from damage caused by isolating the protein.

A striking property of ferricytochrome *c′* is its pH-dependent optical transition with a p*K* of 7–9 [5,31]. This arises from the ionisation of its axial histidine ligand to histidinate [32,33], a property shared with the class IIb cytochromes *c* [5] and *E. coli* cytochrome *b*-562 [34]. The origin of the variation in p*K* for cytochromes *c′* of different organisms is not clear, but the fact that there is a difference may be relevant to studies of ligand binding. For example, Yoshimura et al. [35,36], following the original study of Taniguchi and Kamen [37], have studied NO binding to a variety of ferricytochromes *c* at pH 7.2. They found that with the *Alcaligenes* (NC1B 11015) protein reductive nitrosylation occurred, resulting in a 5-coordinate NO-ferrous cytochrome, whereas with the *Rb. capsulatus* protein a 6-coordinate NO-ferric protein with an intact Fe-His band was produced. These results are probably related to the fact that in the *Alcaligenes* protein the axial histidine ionises with a p*K* of about 7.1 [38], but in the *Rb. capsulatus* protein it has a p*K* of about 8.4 [30].

Since cytochrome *c′* operates in the periplasmic space, which has a pH < 7, it is unlikely that the histidine ionisation has a major physiological significance, unless it is perturbed in situ [30]. Therefore, the functional form of cytochrome *c′* will have a mono-His-coordinated haem. This has relatively slow ligand binding properties [3,39,40] and this, coupled with its relatively low redox potential, of −10 to 130 mV (Ref. 5 and references therein), makes it unlikely that cytochrome *c′* is a terminal oxidase. Therefore cytochrome *c′* probably functions as an electron transfer protein. Despite theoretical concerns that the high-spin state of the iron may produce a poor intrinsic electron transfer reactivity of cytochrome *c′* [5], Meyer et al. [41] have shown that, in its reaction with photoreduced flavins, ferricytochrome *c′* has a rate of reaction that correlates well with those of low-spin cytochromes with similar redox potentials.

Bacterioferritin and haemoferritin

Bacterioferritin resembles animal ferritin in many respects: both are approximately spherical proteins of about 120 Å diameter consisting of 24 subunits of about 20 kDa, and both contain a central cavity of about 80 Å diameter into which a non-haem iron core can be deposited [42]. A major difference between the two proteins is that, as isolated, animal ferritin does not contain haem, though bacterioferritin does. The ratio of haem to protein subunits in the isolated bacterioferritin is variable, but addition of haemin chloride results in a haem : subunit ratio of 1 : 1 [43].

A variety of questions are raised by the presence of haem in bacterioferritin. For example: if the role of the haem is in iron uptake and/or release why does animal ferritin not contain haem? Partly because of the lack of haem in animal ferritin, other functions have been sought for bacterioferritin. For example, the protein appears [44] to be the same as the cytochrome b_1 isolated by Deeb and Hager [45], and shown by them to be involved in respiratory redox reactions: therefore, is bacterioferritin a respiratory protein? Another possibility is that bacterioferritin acts as an electron store and the haem is required to assist the passage of electrons through the protein shell. Recently published data suggest that neither of these latter suggestions is correct.

Respiratory proteins tend to be located in the periplasmic space of bacteria or are transmembraneous. Both locations require a signal sequence to enable the protein to be transferred from the cytoplasm. However, since the gene sequence and amino-acid sequence of *E. coli* bacterioferritin [22,46] reveal that this protein does

not contain a signal sequence, it is almost certainly cytoplasmic and hence not a respiratory protein. ^{57}Fe Mössbauer spectroscopy of intact *Ps. aeruginosa* cells indicates that the iron core of bacterioferritin in situ is largely in the Fe^{3+} state [47,48]. Hence it is not an electron store. These considerations lead back to the idea that the haem of bacterioferritin is involved in iron storage, emphasising again the question of why animal ferritin does not contain haem?

One reason may be because there is a haemoprotein reaction partner for ferritin [49], but another, and in some ways more attractive, possibility is that animal ferritin does require a haem in situ but that this haem is lost on isolation of the ferritin or as a non-haem iron core is laid down. We investigated the binding of haem to horse spleen ferritin and found that up to 16 haems per 24 subunits were bound, each in a pocket containing two strong-field axial ligands [50]. This latter is indicated by the low-spin character of the ferritin bound haem. We have termed the haem-ferritin complex haemoferritin to emphasise that haem affects a key mechanistic feature of ferritin: it increases the rate of iron release in an in vitro assay system [51]. Thus the haem is not merely adventitiously bound.

The 4-α-helical class of cytochromes

A classification scheme for proteins is useful if it highlights some evolutionary or mechanistic relationship. In the present case, despite attempts to show that cytochrome *c′* and cytochrome *b*-562 have a common evolutionary relationship [13], when all the proteins are considered together it seems unlikely that they have a common origin. The differences in their iron axial ligations automatically produces large differences in spectroscopic and chemical properties. Therefore, the only basis for considering these proteins together is that they have a common protein fold; but so do tobacco mosaic virus and hemerythrin [12], both non-haem proteins. Thus, it seems to the present author that only if all 4-α-helical bundle proteins turn out to be capable of binding haem will it be useful to categorise the proteins of Table I as a family of cytochromes comparable to other structural families.

Acknowledgements

I wish to thank the Wellcome Trust and the SERC for supporting my work on ferritins and cytochromes, and my colleagues and students at UEA, particularly Drs. F.H.A. Kadir, A.G. McEwan and M.R. Cheesman, Professors A.J. Thomson and C. Greenwood, Mrs. F. Al-Massad and Messrs. S.J. Bingham and F.A. Monkara, for their help in establishing the stories concerning cytochrome *c′* and ferritin discussed above.

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