Note to the Editor

Further evidence for structural homology of pyruvate ferrodoxin oxidoreductases: sedimentation velocity behaviour

S. E. Harding
University of Nottingham, Department of Applied Biochemistry and Food Science, Sutton Bonington LE12 5RD, UK

and K. P. Williams
University of Cambridge, Department of Biochemistry, Tennis Court Road, Cambridge CB2 1QW, UK
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Evidence has recently been presented for physicochemical homology between pyruvate:ferrodoxin oxidoreductases (PFORs). We support this homology by examining specifically the sedimentation velocity behaviour of PFOR from T. vaginalis *(both sedimentation coefficient and Fe-S cluster co-sedimentation behaviour) in relation to data for PFORs from other species.*

Keywords: Oxidoreductases; sedimentation velocity

Evidence has recently been presented[1] for close homology between pyruvate:ferrodoxin oxidoreductase (PFOR) from the protozoan *Trichomonas vaginalis* and PFORs from bacterial species: (*Clostridium acidiurici*[2] and *Halobacterium halobium*[3]), and also a pyruvate flavodoxin oxidoreductase from *Klebsiella pneumoniae*[4]. This evidence for homology was derived from measurement of enzyme activity, cofactor content, relative molecular mass and related subunit composition. Physicochemical homology was judged from calibrated gel chromatography and low speed sedimentation equilibrium (for native M_r determination) and SDS polyacrylamide gel electrophoresis (dimeric composition; but see Ref. 1 for a discussion on the composition of *Halobium* PFOR). Wahl and Orme-Johnson[5] have also observed close homology between PFORs from *K. pneumoniae*, *C. thermoaceticum* and *C. acidiurici*, based on chemical, spectrophotometric and e.s.r. studies. We now provide further support for the homology of the *T. vaginalis* PFOR with the enzymes from other species from its sedimentation velocity behaviour in the analytical ultracentrifuge.

PFOR from *Trichomonas vaginalis* was extracted and purified as described by Williams *et al.*[1]. The native enzyme was suspended in buffer ($I = 0.42$, pH = 7.4) containing 20 mM Tris–HCl, 0.4 M KCl, 1 mM EDTA, 2 mM dithiothreitol and 1 mM $Na_2S_2O_4$. The high salt concentration was required to prevent aggregation and precipitation. The sample was deemed pure and monodisperse by using the sensitive technique of SDS PAGE.

Sedimentation velocity was performed with an MSE Centriscan analytical ultracentrifuge, rotor speed 35 000 rev/min, temperature 20°C. Because of the scarcity of the material and to minimize possible concentration dependence effects a very low concentration (~0.2 mg/ml) was used but sufficient to register a measurable trace on the output with scanning u.v. absorption optics at 280 mm (*Figure 1*). Standard MSE 10-mm pathlength cells were employed. The sedimentation data were captured by using a Cherry digitizing tablet interfaced to an Apple IIE computer which evaluated the sedimentation coefficient and the radial dilution correction factor for concentration.

The scans (*Figure 1*) produced correspond to a single-solute species with no evidence of any dissociation products or aggregates, confirming the purity of the preparation. A value for the sedimentation coefficient $s_{20,w}$ (after correction to standard conditions (water at 20.0°C)) of $(10.1 \pm 0.1) \times 10^{-13}$ s was obtained.

Figure 1 also shows a scan at 400 nm taken shortly (~60 s) after the last of the 280 nm scans; the point of inflection of the sedimenting boundary corresponds very closely to that of the enzyme (at 280 nm) confirming co-sedimentation of the iron–sulphur clusters with the enzyme.

Our measured $s_{20,w}$ value for PFOR from *T. vaginalis* is in good agreement with other published values for PFORs from different sources. For example, Uyeda and Rabinowitz[2] obtained an (infinite dilution) value of 9.4×10^{-13} s for PFOR from *Clostridium acidiurici*. From their absorbance measurements at 400 nm this component also appears to have the Fe–S clusters present. These workers also observe a small trailing minor component (using schlieren optics) of $s_{20,w} = 5.6 \times 10^{13}$ s; the presence of this could possibly have contributed to the slightly lower value they obtain for the pure enzyme (9.4×10^{-13}) compared with the present study (faster

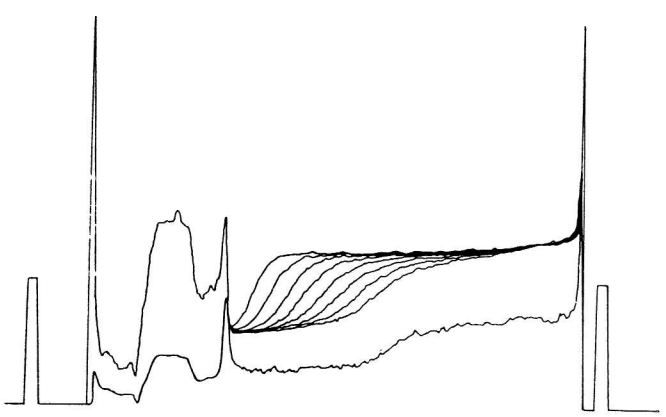

Figure 1 Sedimentation velocity scans (at 10-min time intervals) obtained with absorption optics, of purified *Trichomonas vaginalis* PFOR. Top scans at 280 nm. Lower scan (with signal amplified 4×) at 400 nm taken ~60 s after last 280-nm scan. Other details as text. The direction of sedimentation is from left to right

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component moving through solution of slower component, see, e.g. Ref. 7).

Kerscher and Oesterhelt[3] obtain for the PFOR from *Halobacterium halobium* an s_{20} value of 5.8×10^{-13} s in solvent containing 3.0 M KCl at low enzyme concentration ($\lesssim 1$ mg/ml, and with no observable concentration dependence in this region). After correction to standard conditions as above, we obtain from their data a value for $s_{20,w}$ of 9.5×10^{-13} s, again in good agreement for the other PFORs. These workers also observe co-sedimentation of the Fe-S clusters at 400 nm. Finally, Gehring and Arnon[6] obtain a value of $(10.7 \pm 0.1) \times 10^{-13}$ s using calibrated sucrose density gradient centrifugation for a PFOR from *C. thiosulfatophilum*.

Present evidence suggests that PFOR is an ancient enzyme and already existed before the divergence of the urkingdoms[8]. The results presented here support a close physicochemical homology between the bacterial and eukaryotic PFORs; it would be of considerable interest now to see how closely related the PFORs from archaebacteria and *Clostridia* are with the *Trichomonal* enzyme in terms of sequence homology.

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