Sedimentation Studies of Cytochrome c

ANDERS EHRENBERG and SVEN PALÉUS

Medicinska Nobelinstitutet, Biokemiska avdelningen, Stockholm, Sweden

Pedersen [1] more than 15 years ago determined the sedimentation constant ($S°_{20} = 1.83 \times 10^{-13}$ *s*) and the diffusion coefficient ($D°_{20} = 11.3 \times 10^{-7}$ $cm^2 \cdot s^{-1}$) of the purified cytochrome c obtained by Theorell and Åkeson [2] from beef heart. Recently, Atlas *et al.*[3] reported that they had determined corresponding data of cytochrome c from different sources. Their values have been quoted by Edsall [4] as $S°_{20}$ about 2.3×10^{-13} *s* and $D°_{20}$ about 13×10^{-7} $cm^2 \times s^{-1}$ for cytochrome c from beef, horse and pig. Because of these discrepancies and the modified preparation methods developed [5,6], we found that a more systematic investigation would be of interest.

The sedimentation studies were made with a Spinco analytical ultracentrifuge model E, using a synthetic boundary cell, which is especially suited for work on slowly sedimenting molecules. The reliability of the method was controlled by running bovine albumin (Armour fraction V) both in the common and the synthetic boundary cell. This substance has previously been used in a comparative study of values obtained by a Spinco centrifuge and an oil turbin centrifuge [7]. The values thus obtained agreed fully with these data with a discrepancy less than 1 %. All our runs on cytochrome preparations were made at 260 000 g, and 0.05 *M* phosphate buffer of pH 6.8 with 1 % sodium chloride added was consequently used. Protein concentrations between 1 and 5 mg/ml were used, and in none of the cases could any concentration dependence of $S°_{20}$ be seen.

In a few cases we tried to determine the diffusion coefficient at 23°, using a Tiselius apparatus with the Philpot-Svensson optical system, and a cell 15 mm long and 2 mm broad. An interference filter (Bausch & Lomb) after the lamp allowed a red light of 639 mμ so that the image on the plate was homogeneously illuminated. Because of the low illumination, exposures of several minutes were necessary. The diffusion coefficients were calculated according to the "height-area" method. The percentage of error of our data appeared to be as high as ±10 %, mostly due to an imperfect initial boundary and disturbances during the compensation procedure in the narrow cell.

For salmon and chicken cytochrome the apparent partial specific volume was determined both in water and in the buffer, by means of a common pycnometer. No significant influence of the buffer could be

Table 1.

Material	Preparation method	% Fe	$S°_{20} \times 10^{13}$	Runs in the centrifuge	$D°_{20} \times 10^{7}$	V_p	f/f_0
Beef	P &N [5]	0.42	1.64±0.01	4	(11.3) [1]	(0.707) [2]	1.25
Horse	P &N	0.41	1.89±0.15	2	9.5	(0.707)	1.34
Horse	L &B [6]	0.31	1.61±0.06	2			1.41
Horse	L &B	0.36	1.61±0.02	2			1.41
Horse	L &B	0.36	1.55	1			1.43
Salmon	P &N *	0.41	2.33±0.15	3	10.2	(0.707)	1.19
Salmon	P &N	0.45	2.19±0.15	3			1.22
Salmon	P &N	0.48	1.76±0.10	6			1.31
Salmon	L &B	0.27	1.50±0.02	6	10.7	0.75	1.25
Salmon	L &B	0.27	1.45±0.10	3			1.26
Chicken	P &N	0.37	1.63±0.03	8	(11.3)	0.72	1.23

* Some denaturation might have occurred in this preparation.

seen. Our results are summarized in Table 1. The errors given for $S°_{20}$ are the calculated standard deviations of the mean of each preparation.

It is seen that $S°_{20}$ varies both between the different species and between the two preparation methods for the same species. In spite of their lower iron content, the preparations according to Loftfield and Bonnichsen give lower $S°_{20}$ values than those according to Paléus and Neilands. This indicates that the former preparations might contain impurities with slightly lower sedimentation constant than cytochrome c itself. The two components cannot be resolved but should give rise to a higher apparent diffusion constant which, however, could not be detected in the ultracentrifuge. The high value of V_p for salmon cytochrome c according to Loftfield and Bonnichsen might also be due to this impurity.

In only two cases we got sedimentation constants comparable to those of Atlas *et al.*[3], but one of them might have been modified by partly denaturation.

Our value for beef cytochrome c is 10 % lower than the cited value of Pedersen. This is in accord with the known difference of 5 to 10 % between the oil turbin and the Spinco centrifuge [7, 8].

The calculated frictional ratios also agree quite well with the older value 1.29 of beef cytochrome but not with the data of Atlas *et al.* close to 1.0. The rather high values of f/f_0 for horse cytochrome c might reflect that the diffusion constant of 9.5×10^{-7} cm^2 $\times$ s^{-1} is somewhat too low.

1. Pedersen, K. O. quoted by Paul, K. G. in Sumner, J. B. and Myrbäck, K. *The Enzymes*, Vol. II, Part 1, Academic Press Inc., New York, 1951, p. 375.
2. Theorell, H. and Åkeson, Å. *J. Am. Chem. Soc.* **63** (1941) 1804.
3. Atlas, S. M., Farber, E. and Stern, K. G. *122 Meeting of the Am. Chem. Soc.* Atlantic City, Sept. 1952, p. 23c.
4. Edsall, J. T. in Neurath, H. and Bailey, K. *The Proteins I*, Academic Press Inc., New York 1952, p. 634.
5. Paléus, S. and Neilands, J. B. *Acta Chem. Scand.* **4** (1950) 1024.
6. Margoliash, E. *Nature* **170** (1952) 1014.
7. Shulman, S. *Arch. Biochem. Biophys.* **44** (1953) 230.
8. Loftfield, R. B. and Bonnichsen, R., quoted by Boeri, E. and Tosi, L. *Arch. Biochem. Biophys.* **52** (1954) 83.
9. Pedersen, K. O. in *Les Protéines, Rapports et Discussions, 9ème conseil de chimie, Institut International de Chimie Solvay, 1953, p. 1.*

Received March 11, 1955.

Effects of X-rays and Water Content on Sugars in Barley Seeds

L. EHRENBERG and M. JAARMA

Institute of Organic Chemistry and Biochemistry, University, Stockholm, Sweden

An investigation of the influence of ionizing radiations on biochemical systems demonstrated that the relative concentrations of simple sugars in growing *Vicia faba* plants were strongly affected by chronic γ-irradiation [1]. When resting (*i. e.*, ripe) barley seeds are irradiated, their radiosensitivity was found to decrease with increasing water content [2]. Since the concentration of reducing sugars has been shown [3,4] to increase with increasing moisture content, we found it important to investigate possible relations between sugar content and radiation sensitivity. Under certain conditions glucose may act as a protective agent [5].

Methods. Seeds of the two-rowed barley strain, Bonus, were treated for 6 days with streaming air of the relative humidities 0 % and 90 %. This treatment gave samples containing 7.5 % and 17 % H_2O, respectively. The seeds were irradiated with 175 kV unfiltered X-rays at an intensity of 4 000 r min.$^{-1}$. Analysis of reducing sugars was made 15 hours after irradiation, and again after 2—4 weeks' storage at a constant water content and at 20° C.

For the analysis the material was ground twice in a Wiley mill, using first a 20-mesh, then a 40-mesh sieve. The grist was boiled for 3 min. in 80% ethanol, and thereafter extracted, with the same solvent, for 3 hours in a micro-Soxhlet apparatus. (Using 96 % ethanol instead, only about two thirds of the sugars were extracted by the same procedure.) After deproteinizing [6], reducing sugars were titrated, with an accuracy of 2 %, according to Somogyi [7]. The maximum variation between individual extractions amounted to 5 %.

Results. It was demonstrated (Table 1) that in the unirradiated material the in-