The Amino Acid Sequence of Cytochrome *c* of *Fagopyrum esculentum* Moench (Buckwheat) and *Brassica oleracea* L. (Cauliflower)

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The amino acid sequences of buckwheat and cauliflower cytochromes *c* were determined on $1\frac{1}{2}$ μ mol and 1 μ mol of protein respectively. The molecules consist of 111 residues and are homologous with other plant mitochondrial cytochromes *c*. Experimental details are given in a supplementary paper that has been deposited as Supplementary Publication SUP 50 005 at the National Lending Library for Science and Technology, Boston Spa, Yorks. LS23 7BQ, U.K., from whom copies can be obtained on the terms indicated in *Biochem. J.* (1971), 121, 7.

The determination of the amino acid sequences of buckwheat and cauliflower cytochrome *c* is a continuation of the investigation of the sequences of a wide range of plant cytochromes *c* (Boulter, Thompson, Ramshaw & Richardson, 1970).

MATERIALS

Buckwheat seeds were obtained from the Wearside Seed Co. Ltd., Sunderland, Co. Durham, U.K., and cauliflowers from Crawfords Ltd., Durham City, U.K. Other materials were as previously described (Thompson, Richardson & Boulter, 1971a).

METHODS

Cytochrome *c* from buckwheat was isolated and purified as described by Richardson, Laycock, Ramshaw, Thompson & Boulter (1970) and from cauliflower as described by Richardson, Richardson, Ramshaw, Thompson & Boulter (1971). The E_{350} (reduced)/ E_{280} (oxidized) ratios were 1.10 and 1.22 for buckwheat and cauliflower respectively.

The methods used for the determination of the amino acid sequence were as previously described (Thompson *et al.* 1971a; Ramshaw, Thompson & Boulter, 1970).

RESULTS

The amino acid compositions of buckwheat and cauliflower cytochrome *c* are given in Table 1. The complete sequence of these cytochromes have been determined (see Figs. 1 and 2 respectively).

Consideration of the mobilities of the peptides obtained by enzymic digestion indicated that there were 11 amide groups in the sequence of buckwheat, and ten in cauliflower. In buckwheat three of these

were placed by reference to other cytochrome *c* sequences. Thus reference to all other known cytochrome *c* sequences suggests that position 24 is glutamine, and that glutamic acid occurs in position 98, indicating that glutamine occupies position 97. Homology with other plant cytochrome *c* sequences indicates that position 5 is acidic and hence that asparagine occurs in position 10. One of the two positions 108 and 111 contains an amide residue, but present evidence does not indicate which one.

In cauliflower cytochrome *c* there are three amide positions that cannot be placed by peptide mobilities. Of these, reference to all other cytochromes *c* suggests that glutamine occurs in position 24, so position 29 is therefore aspartic acid, and a similar argument suggests that position 98 is aspartic acid, placing glutamine in position 97. Similarly, by homology with plant cytochromes *c*, position 5 is glutamic acid, and thus position 4 or 10 is an amide.

DISCUSSION

Some 34 residues have so far been found to be invariant in all cytochrome *c* sequences determined to date (Dayhoff, 1969; Boulter *et al.* 1970; Thompson *et al.* 1971a; Thompson, Richardson & Boulter, 1971b; Thompson, Notton, Richardson & Boulter, 1971c). The determination of the sequences for buckwheat and cauliflower cytochromes *c* does not change this number. Both sequences have the features typical of higher-plant cytochromes, namely an acetylated 'tail' of eight residues as compared with mammalian cytochrome *c*, two residues of trimethyl-lysine, and threonine in both positions 50 and 51.

Table 1. *Amino acid composition of buckwheat and cauliflower cytochromes c*

The values for the amino acid analysis are the averages for three 24 h and one 72 h hydrolysates. Abbreviations: n.d., not determined; Me₃Lys, ε-trimethyl-lysine.

	Amino acid composition (residues/molecule)			
	Buckwheat cytochrome c		Cauliflower cytochrome c	
	Amino acid analysis	Calc. from sequence	Amino acid analysis	Calc. from sequence
Hyp	—	—	1.82	—
Asp	9.96	9	11.23	11
Thr	8.70*	9	7.04*	7
Ser	5.28*	6	5.82*	5
Glu	9.52	11	8.44	10
Pro	5.83	7	7.86	7
Gly	12.00	12	10.31	11
Ala	8.86	10	11.30	12
Val	4.41†	3	4.22†	3
Met	1.35	1	1.86	1
Cys	n.d.	2	2.53	2
Ile	4.05†	4	3.06†	3
Leu	7.78	8	7.96	8
Tyr	5.60	6	5.35	6
Phe	4.03	4	3.62	4
Me ₃ Lys	1.70	2	1.67	2
Lys	11.60	12	11.81	13
His	2.27	2	1.70	2
Arg	2.08	2	3.10	2
Trp	n.d.	1	n.d.	1

*Calculated assuming first-order kinetics for destruction.

† Maximal yield.

Fig. 1. Amino acid sequence of buckwheat cytochrome c. ---, Composition determined qualitatively; —, composition determined by quantitative amino acid analysis; —, sequence determined by the dansyl-Edman method; —, sequence determined by using carboxypeptidase; ↓, major enzymic cleavages; ↓, partial enzymic cleavages; Abbreviations: Me₃Lys, ε-trimethyl-lysine; T, tryptic; C, chymotryptic. For full details of individual peptides, see the supplementary paper SUP 50005.

Buckwheat belongs to the Polygonaceae, generally considered to be closely related to the Caryophyllales, also known as the Centrospermae.

In earlier classifications such as those of Bentham and Hooker, the Polygonaceae are placed in the primitive 'Monochlamydeae', which is now gener-

Fig. 2. Amino acid sequence of cauliflower cytochrome *c*. The abbreviations and symbols used are described in the legend of Fig. 1.

ally considered to be an heterogonous group (Takhtajan, 1969, p. 97). The sequence results presented here clearly distinguish buckwheat cytochrome *c* from other sequences of cytochromes *c* from dicotyledons (see Fig. 3 of Thompson *et al.* 1971c), and this is in accord with modern views of taxonomists, such as Takhtajan (1969, Fig. 31 on p. 239). The exact placing of buckwheat on the phylogenetic 'tree' must await the determination of sequences of cytochrome *c* of other plants, especially members of the Ranunculales and Magnoliales.

Cauliflower is a member of the Capparales, and the sequence of its cytochrome *c* is most closely related to that of pumpkin, a member of the Cucurbitales (see Fig. 3 of Thompson *et al.* 1971c); this accords with modern taxonomic opinion (Takhtajan, 1969, Fig. 31 on p. 239).

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REFERENCES

- Boulter, D., Thompson, E. W., Ramshaw, J. A. M. & Richardson, M. (1970). *Nature, Lond.*, **228**, 552.
 Dayhoff, M. O. (1969). *Atlas of Protein Sequence and Structure*, pp. D8-D20. Silver Spring, Md.: National Biomedical Research Foundation.
 Ramshaw, J. A. M., Thompson, E. W. & Boulter, D. (1970). *Biochem. J.* **119**, 535.
 Richardson, M., Laycock, M. V., Ramshaw, J. A. M., Thompson, E. W. & Boulter, D. (1970). *Phytochemistry*, **9**, 2271.
 Richardson, M., Richardson, D. L., Ramshaw, J. A. M., Thompson, E. W. & Boulter, D. (1971). *J. Biochem., Tokyo*, **69**, 811.
 Takhtajan, A. (1969). *Flowering Plants—Origin and Dispersal*. Edinburgh: Oliver and Boyd.
 Thompson, E. W., Notton, B. A., Richardson, M. & Boulter, D. (1971c). *Biochem. J.* **124**, 787.
 Thompson, E. W., Richardson, M. & Boulter, D. (1971a). *Biochem. J.* **121**, 439.
 Thompson, E. W., Richardson, M. & Boulter, D. (1971b). *Biochem. J.* **124**, 779.