- Ambler, R. P. (1974) *Biochem. J.* **137**, 3–14
- Ambler, R. P. (1975) *Biochem. J.* **151**, 197–218
- Ambler, R. P. & Bartsch, R. G. (1975) *Nature (London)* **253**, 285–288
- Ambler, R. P. & Taylor, H. E. (1973) *Biochem. Soc. Trans.* **1**, 166–168
- Ambler, R. P. & Wynn, M. (1973) *Biochem. J.* **131**, 485–498
- Bartsch, R. G. (1976) in *The Photosynthetic Bacteria* (Clayton, R. K. & Sistrom, W. R., eds.), Plenum Press, New York, in the press
- Clayton, R. K. (1963) in *Bacterial Photosynthesis* (Gest, H., San Pietro, A. & Vernon, L. P., eds.), pp. 495–500, Antioch Press, New York
- Cusanovich, M. A. & Bartsch, R. G. (1969) *Biochim. Biophys. Acta* **189**, 245–255
- Davis, K. A., Hatefi, Y., Salemm, F. R. & Kamen, M. D. (1972) *Biochem. Biophys. Res. Commun.* **49**, 1328–1335
- Edman, P. & Begg, G. (1967) *Eur. J. Biochem.* **1**, 80–91
- Fowler, C. F., Nugent, N. A. & Fuller, R. C. (1971) *Proc. Natl. Acad. Sci. U.S.A.* **68**, 2278–2282
- Gibson, J. (1961) *Biochem. J.* **79**, 151–158
- Gray, B. H., Fowler, C. F., Nugent, N. A. & Fuller, R. C. (1972) *Biochem. Biophys. Res. Commun.* **47**, 322–327
- Gray, B. H., Fowler, C. F., Nugent, N. A. & Fuller, R. C. (1973) *Int. J. Syst. Bacteriol.* **23**, 256–264
- Kamen, M. D. & Vernon, L. P. (1954) *J. Bacteriol.* **67**, 617–618
- Keil-Dlouhá, V., Zylber, N., Imhoff, J.-M., Tong, N.-T. & Keil, B. (1971) *FEBS Lett.* **16**, 291–295
- Larsen, H. (1967) *Adv. Microbial Physiol.* **6**, 97–132
- Mandel, M., Leadbetter, E. R., Pfennig, N. & Trüper, H. G. (1971) *Int. J. Syst. Bacteriol.* **21**, 222–230
- Meyer, T. E. (1970) Ph.D. Thesis, University of California, San Diego
- Meyer, T. E., Bartsch, R. G., Cusanovich, M. A. & Mathewson, J. H. (1968) *Biochim. Biophys. Acta* **153**, 854–861
- Nelson, N. & Racker, E. (1972) *J. Biol. Chem.* **247**, 3848–3853
- Olson, J. M. (1973) *Int. J. Syst. Bacteriol.* **23**, 265–266
- Olson, J. M. & Shaw, E. K. (1969) *Photosynthetica* **3**, 288–290
- Olson, J. M., Philipson, K. D. & Sauer, K. (1973) *Biochim. Biophys. Acta* **292**, 206–217
- Penke, B., Ferenczi, R. & Kovacs, B. (1974) *Anal. Biochem.* **60**, 45–50
- Perini, F., Kamen, M. D. & Schiff, J. A. (1964) *Biochim. Biophys. Acta* **88**, 74–98
- Pfennig, N. & Trüper, H. G. (1974) in *Bergey's Manual of Determinative Bacteriology*, 8th edn. (Buchanan, R. E. & Gibbons, N. E., eds.), pp. 24–75, Williams and Wilkins, Baltimore
- Prince, R. C. & Olson, J. M. (1976) *Biochim. Biophys. Acta* **423**, 357–362
- Salemm, F. R., Freer, S. T., Xuong, Ng. H., Alden, R. A. & Kraut, J. (1973) *J. Biol. Chem.* **248**, 3910–3921
- Shioi, Y., Takamiya, K. & Nishimura, M. (1972) *J. Biochem. (Tokyo)* **71**, 285–294
- Swank, R. T. & Burris, R. H. (1969) *Biochim. Biophys. Acta* **180**, 473–489
- Van Beeumen, J. & Ambler, R. P. (1973) *Antonie van Leeuwenhoek J. Microbiol. Serol.* **39**, 355–356
- Van Niel, C. B. (1957) in *Bergey's Manual of Determinative Bacteriology*, 7th edn. (Breed, R. S., Murray, E. G. D. & Smith, N. R., eds.), pp. 62–63, Williams and Wilkins, Baltimore
- Yamanaka, T. & Okunuki, K. (1968) *J. Biochem. (Tokyo)* **63**, 341–346
- Yamanaka, T., Takenami, S., Akiyama, N. & Okunuki, K. (1971) *J. Biochem. (Tokyo)* **70**, 349–358

APPENDIX

Comparison of Bacteriochlorophyll *a*-Proteins from Two Green Bacteria

By JOHN M. OLSON, ELIZABETH K. SHAW and FRIEDA M. ENGLBERGER

Department of Biology, Brookhaven National Laboratory, Upton, NY 11973, U.S.A.

(Received 13 May 1976)

Bacteriochlorophyll *a*-proteins have been obtained from three strains of green bacteria, namely *Chlorobium limicola* 2K, *Chlorobium thiosulphatophilum* L and *C. thiosulphatophilum* 6230 (Tassajara) (Olson & Romano, 1962; Olson *et al.*, 1973). The best characterized bacteriochlorophyll *a*-protein is that from *C. limicola* 2K, which when grown in mixed culture with one or more non-photosynthetic sulphate-reducing bacteria has been known as '*Chloropseudomonas ethylica* 2K' (Gray *et al.*, 1973; Olson, 1973). The bacteriochlorophyll *a*-protein from strain 2K is a trimer (Fenna *et al.*, 1974), composed solely of bacteriochlorophyll *a* and protein of known composition (Thorner & Olson, 1968). Each subunit con-

sists of a protein bag (354 residues) with seven chlorophyll molecules inside the bag (Fenna & Matthews, 1975, 1976). Since the absorption and circular-dichroism spectra at 77K are distinctly different for bacteriochlorophyll *a*-proteins from strains 2K and Tassajara (Olson *et al.*, 1973, 1976), we wished to find out whether these spectral differences might be correlated with biochemical differences between the proteins.

Experimental*Materials*

Chlorobium limicola 2K was grown in pure culture (Gray *et al.*, 1973) or in mixed culture as

'*Chloropseudomonas ethylica* 2K' (Bose, 1963) and harvested by alum precipitation (Thornber & Olson, 1968). *C. thiosulphatophilum* 6230 (Tassajara) was grown and harvested similarly (Olson *et al.*, 1973). [*C. thiosulphatophilum* is called '*C. limicola* forma sp. *thiosulphatophilum*' by Pfennig & Trüper (1974).] Bacteriochlorophyll *a*-proteins were prepared as described by Olson (1971).

Methods

Electrophoresis. To check the presence of impurities in bacteriochlorophyll *a*-protein preparations, polyacrylamide-gel electrophoresis was carried out. Some samples were electrophoresed in slab gels (10 or 20%, w/v, acrylamide) in 0.15M-Tris/HCl, pH 8.8, and 2 mM-EDTA (Studier, 1973), whereas others were tested in tube gels (6mm×10cm; 7.5%, w/v, acrylamide) in 5mM-Tris/HCl, pH 9.0, and 8M-urea.

Isoelectric focusing. Isoelectric focusing was carried out with 5% (w/v) polyacrylamide gels incorporating the desired ampholytes (Righetti & Drysdale, 1971). If 0.1–0.2mg of bacteriochlorophyll *a*-protein/tube was loaded, bands containing bacteriochlorophyll *a* could be observed before staining.

Amino acid analyses. Samples of crystalline or highly purified non-crystalline bacteriochlorophyll *a*-proteins that had been checked by electrophoresis were weighed to determine the amount of protein (86%) in each sample. Approx. 1.5mg of bacteriochlorophyll *a*-protein was hydrolysed with 4M-methanesulphonic acid for tryptophan determination by the method of Liu & Chang (1971). Hydrolysis was carried out at 115°C in sealed evacuated tubes for 24–96h. An equivalent weight of bacteriochlorophyll *a*-protein was oxidized with performic acid (Hirs, 1967) and hydrolysed with constant-boiling HCl for 24h. The amino acid contents of the hydrolysates were determined on an automatic analyser (Spackman *et al.*, 1958; Alonzo & Hirs, 1968). Best values for tryptophan, serine and threonine content were obtained by extrapolation to zero time. Best values for isoleucine and valine content were obtained by extrapolation to infinite time of hydrolysis. Half-cystine values were derived from the analyses of samples oxidized with performic acid (Thornber & Olson, 1968).

Results

Subunit size

The bacteriochlorophyll *a*-protein from strain 2K was denatured by heating at 100°C for 2min in the presence of 1% sodium dodecyl sulphate and 1% 2-mercaptoethanol, and then electrophoresed on a discontinuous polyacrylamide gel (10%, w/v) in the presence of marker proteins from bacteriophage T7 (Studier, 1972). The molecular weight of the subunit polypeptide was estimated as 40000±2000. The subunit polypeptide from strain Tassajara has the

same molecular weight. When the two proteins were mixed together, heated in sodium dodecyl sulphate and mercaptoethanol, and electrophoresed on polyacrylamide gel, they migrated as a single component.

Isoelectric point

When any sample was focused isoelectrically, 5–13 bands appeared. Since the focusing patterns were not reproducible, and multiple bands always appeared, it was necessary to plot the distribution of bands from each protein. For bacteriochlorophyll *a*-protein from strain 2K there were 17 experiments with 148 bands distributed between pH 5.3 and 7.4. The distribution was centred at pH 6.0. For strain Tassajara there were eight experiments with 53 bands distributed between pH 6.1 and 7.8. This distribution was centred at pH 7.0. The clear difference of 1 pH unit between the 'average' isoelectric values for the two proteins suggested a significant difference in the nature of surface amino acid residues.

Composition

Thornber & Olson (1968) showed that the bacteriochlorophyll *a*-protein from strain 2K consists of bacteriochlorophyll *a* and protein only. Alanine is the *N*-terminal amino acid. In the present study no significant differences in amino acid composition were observed between bacteriochlorophyll *a*-protein samples from pure cultures and samples from mixed cultures. We also compared hydrolysis of bacteriochlorophyll *a*-protein samples in 4M-methanesulphonic acid with hydrolysis in constant-boiling HCl. There were no significant differences, except in tryptophan content, resulting from the method of sample hydrolysis. The average results are compared with those of Thornber & Olson (1968) in Table 1. There are significant differences for three residues, namely histidine (7%), tryptophan (–7%), and half-cystine (9%). The differences in values for tryptophan content may be due to the difference in the methods used. The older value was obtained from a spectrophotometric determination, whereas the new value was obtained by chromatography. The new value is probably more nearly correct. The reason for differences in values for histidine and half-cystine contents is not known.

The amino acid analysis of bacteriochlorophyll *a*-protein from strain Tassajara is given in Table 2, and the results are compared with those for strain 2K in Table 1. Significant differences exist between the two proteins for eleven amino acid residues, namely glycine, valine, glutamic acid (glutamine), serine, isoleucine alanine, arginine, proline, threonine, tryptophan and methionine. Especially prominent are the differences between isoleucine (–39%), tryptophan (–30%) and methionine (23%).

Since the polypeptide size is the same for the two subunits, it is assumed that each polypeptide contains

Table 1. Comparison of amino acid analyses

Compositions are given as molar percentages. Each analysis is compared with the average composition computed from the four analyses in the present paper for bacteriochlorophyll α -protein from strain 2K. (Parallel analyses with methanesulphonic acid and HCl were made with the same protein preparation.) Each number in the columns headed by the symbol Δ is the percentage difference between the average value (strain 2K) and the corresponding value listed for the analysis in question. Only differences greater than 6% are significant.

Hydrolysis method	...	Strain 2K*, mixed culture HCl		Strain 2K, average composition		Pure culture		Mixed culture		Strain Tassajara Methane- sulphonic acid					
		HCl				Methane- sulphonic acid		HCl		Methane- sulphonic acid					
		Mol %	Δ (%)	Mol %	Δ (%)	Mol %	Δ (%)	Mol %	Δ (%)	Mol %	Δ (%)				
		Mol %	Δ (%)	Mol %	Δ (%)	Mol %	Δ (%)	Mol %	Δ (%)	Mol %	Δ (%)				
Amino acid															
Gly		11.26	-1	11.43		11.47	0	11.46	0	11.46	0	11.34	-1	10.46	-8†
Asp		10.91	-1	11.01		11.01	0	10.80	-2	11.08	1	11.15	1	11.14	1
Val		9.56	0	9.58		9.56	0	9.68	1	9.39	-2	9.67	1	10.69	12†
Glu		9.23	-2	9.39		9.52	1	9.40	0	9.35	0	9.30	-1	8.48	-10†
Ser		7.53	-3	7.77		8.12	4	7.65	-2	7.59	-2	7.71	-1	9.13	18†
Ile		6.59	0	6.57		6.36	-3	6.56	0	6.64	1	6.72	2	3.98	-39†
Ala		6.01	0	6.04		6.01	0	6.10	1	6.03	0	6.02	0	6.57	8†
Leu		5.72	1	5.65		5.56	-2	5.69	1	5.66	0	5.70	1	5.91	5
Arg		5.84	6	5.52		5.36	-3	5.60	1	5.55	0	5.55	0	6.32	14†
Pro		4.91	-4	5.09		5.31	4	4.98	-2	5.14	1	4.93	-3	5.87	15†
Lys		5.18	3	5.02		5.00	0	5.17	3	4.99	-1	4.90	-2	5.10	2
Phe		4.89	3	4.76		4.69	-1	4.77	0	4.73	-1	4.83	1	4.95	4
Thr		4.09	4	3.92		3.88	-1	3.83	-2	4.06	4	3.90	0	3.34	-15†
Tyr		2.66	-1	2.70		2.67	-1	2.73	1	2.70	0	2.70	0	2.78	3
His		2.26	7†	2.12		2.11	0	2.15	1	2.11	0	2.10	-1	2.13	0
Trp		1.74	-7†	1.88		1.82	-3	—	—	1.94	3	—	—	1.31	-30†
Met		0.99	1	0.98		0.95	-3	0.97	-1	1.00	2	1.01	3	1.21	23†
Cys		0.63	9†	0.58		0.59	2	0.59	2	0.58	0	0.57	-2	0.60	+3
Total		100.00		100.01		99.99		98.13		100.00		98.10		99.97	

* Data from Thorner & Olson (1968).
† Difference greater than 6%.

Table 2. *Amino acid analysis of bacteriochlorophyll a-protein from strain Tassajara*

Protein was hydrolysed in 4M-methanesulphonic acid. The sample volume was 5.0ml.

Amino acid	Hydrolysis time ...	Content (nmol)				Best value
		24h	64h	96h	Ox.*	
Trp		100	96	83	—	102
Lys		386	398	406	374	397
His		164	161	172	150	166
Arg		479	477	519	466	492
Cys		—	—	—	47	47
Asp		906	874	822	910	867
Thr		237	203	179	232	260
Ser		580	414	302	580	710
Glu		676	653	651	675	660
Pro		424	497	449	385	457
Gly		806	818	819	812	814
Ala		516	499	518	505	511
Val		766	795	815	778	832
Met		100	90	93	123	94
Ile†		290	298	305	286	310
Leu		460	458	463	468	460
Tyr		213	206	204	182	216
Phe		386	379	391	386	385
Total						7780

* Sample oxidized with performic acid and hydrolysed for 24h.

† Includes alloisoleucine.

Table 3. *Comparison of bacteriochlorophyll a-proteins*

Amino acid compositions of subunits (mol/mol) are calculated for 354 residues per subunit.

Amino acid	Strain 2K		Strain Tassajara		Minimum difference (nearest integer)
	Mol	Nearest integer	Mol	Nearest integer	
Gly	40.5	39-42	37.0	36-38	-1
Asp	39.0	38-40	39.4	38-41	0
Val	33.9	33-35	37.8	37-39	+2
Glu	33.2	32-34	30.0	29-31	-1
Ser	27.5	26-29	32.3	31-34	+2
Ile	23.3	23-24	14.1	14	-9
Ala	21.4	21-22	23.3	23-24	+1
Leu	20.0	19-21	20.9	20-22	0
Arg	19.5	19-20	22.4	22-23	+2
Pro	18.0	17-19	20.8	20-22	+1
Lys	17.8	17-18	18.0	17-18	0
Phe	16.8	16-17	17.5	17-18	0
Thr	13.9	13-14	11.8	11-12	-1
Tyr	9.6	9-10	9.8	10	0
His	7.5	7-8	7.5	7-8	0
Trp	6.6	6-7	4.6	4-5	-1
Met	3.5	3-4	4.3	4	0
Cys	2.0	2	2.1	2	0
Total	354.0		353.6		-13 +8

at least 354 residues (Fenna & Matthews, 1976). The two subunits can then be compared in terms of minimum differences for each amino acid. In Table 3 this comparison is made for 354 residues. The nearest integer values for each residue are obtained from the experimental values (mol) multiplied by $(1 \pm \text{error})$. The estimated error for all residues except serine, proline and threonine is 3%; for serine, proline and threonine the estimate is 4% (these errors estimated were obtained from the experimental data in Table 1). The minimum difference for any given residue is the difference between the closest integer values for each protein. Since the sum of positive differences is 8, and the sum of negative differences is -13, there must be at least 8-13 residues (approx. 3%) in one polypeptide which differ from the corresponding residues in the other polypeptide.

Molecular weight

From the values of amino acid composition given in Table 1, the molecular weights of the two polypeptides (354 residues, no amide groups) are both calculated to be 38600. With the addition of seven bacteriochlorophyll *a* molecules (6400), the total subunit mol.wt. becomes 45000, and the trimer mol.wt. 135000.

Discussion

The experimental value (40000) for the mol.wt. of the 2K subunit as measured by gel electrophoresis is closer to the calculated value without the seven bacteriochlorophyll *a* molecules included. Within the limitations of accuracy and precision in gel electrophoresis, the true mol.wt. of the denatured subunit could be anywhere between 38000 and 42000. It seems reasonable to assume that the denatured subunit has lost its chlorophyll moiety and therefore has a true mol.wt. near 39000.

The formation of multiple bands during isoelectric focusing of bacteriochlorophyll *a*-proteins is probably due to heterogeneity in the number of amide groups on the surfaces of the macromolecules (Haxo *et al.*, 1976). In the preparation of these proteins the bacteria were stored and broken in the presence of 2M-Na₂CO₃ (pH approx. 10). Under these conditions extensive deamidation and the formation of more acidic forms of bacteriochlorophyll *a*-protein would be expected.

Since the subunit size is the same for the two strains of green bacteria, it is reasonable to assume approximately the same number of amino acid residues in each polypeptide. Other similarities are noteworthy: two cysteine (or half-cystine) residues and seven histidine residues. It is not yet known whether there is an internal S-S linkage in the subunit from strain 2K, or whether there are three linkages between subunits

(Fenna & Matthews, 1975). Three of the histidine residues in the 2K subunit appear to ligand the magnesium atoms of three chlorophyll molecules, but the other four histidines appear not to serve as ligands (Fenna & Matthews, 1976).

When one compares the two strains of green bacteria, the differences between the two bacteriochlorophyll *a*-proteins are as important as the similarities. Underlying biochemical differences have been found which account for the difference in average isoelectric point and which also may account indirectly for the differences in absorption and circular dichroism mentioned in the introduction. The finding that the 'average' isoelectric point for the Tassajara trimer is 1 pH unit above that for the 2K trimer reflects the fact that the Tassajara subunit contains at least two more arginine residues and one less glutamic acid residue than the 2K subunit. Since at least 8-13 (and probably more) residues differ in the two subunits, the relative positions of the seven chlorophyll molecules have to be slightly different in each subunit. This would result in a slightly different exciton interaction among the seven chlorophyll molecules in one subunit as compared with that in the other (Olson *et al.*, 1976).

According to Pfennig & Trüper (1974) *C. thiosulphatophilum* should be classified as a subspecies of *C. limicola* (i.e. *C. limicola* forma sp. *thiosulphatophilum*). However, the amino acid sequence for cytochrome *c*-555 from *C. thiosulphatophilum* (L or PM) differs from the sequence for cytochrome *c*-555 from *C. limicola* 2K in 52 positions out of 99 [see the main paper (Van Beeumen *et al.*, 1976)]. This large difference in sequence suggests that *C. limicola* 2K and *C. thiosulphatophilum* (L or PM) may indeed belong to two distinct species. (The similarities between strains L, PM and Tassajara make it likely that all three belong to one species.) The minimum residue differences between bacteriochlorophyll *a*-proteins from *C. limicola* 2K and *C. thiosulphatophilum* (Tassajara) are in line with the sequence data for cytochromes *c*-555, but more detailed sequence information about the bacteriochlorophyll *a*-proteins is required for an additional criterion by which *C. limicola* 2K and *C. thiosulphatophilum* (L, PM and Tassajara) can be classified.

Note Added in Proof (Received 21 October 1976)

Professor Norbert Pfennig (personal communication) recently identified the organisms in our culture of '*Chloropseudomonas ethylica* 2K' as *Prosthechloris estuarii* and *Desulfomonas acetoxidans* (Pfennig & Biebel, 1976). He found no *Chlorobium limicola* in the culture. If the green bacterium in '*Chloropseudomonas ethylica* 2K' is not *Chlorobium limicola*, then we have to reconsider the possibility that *C. thiosulphatophilum* may be a variety of *C.*

limicola, as indicated by the name introduced by Pfennig & Trüper (1974): '*Chlorobium limicola* forma sp. *thiosulphatophilum*'.

We thank Dr. Teh-Yung Liu and Mr. Nicholas Alonzo for assistance with amino acid analyses, Mrs. Helen Kycia for help with isoelectric focusing, and Mr. Gary McGovern for help with gel electrophoresis. Dr. F. W. Studier determined the molecular weight of the subunit polypeptide from strain 2K. The research was carried out at Brookhaven National Laboratory under the auspices of the U.S. Atomic Energy Commission (now the U.S. Energy Research and Development Administration).

References

- Alonzo, N. & Hirs, C. H. W. (1968) *Anal. Biochem.* **23**, 272-288.
- Bose, S. K. (1963) in *Bacterial Photosynthesis* (Gest, H., San Pietro, A. & Vernon, L. P., eds.), pp. 501-510, Antioch Press, Yellow Springs, OH.
- Fenna, R. E. & Matthews, B. W. (1975) *Nature (London)* **258**, 573-577.
- Fenna, R. E. & Matthews, B. W. (1976) *Brookhaven Symp. Biol.* **28**, in the press.
- Fenna, R. E., Matthews, B. W., Olson, J. M. & Shaw, E. K. (1974) *J. Mol. Biol.* **84**, 231-240.
- Gray, B. H., Fowler, C. F., Nugent, N. A., Rigopoulos, N. & Fuller, R. C. (1973) *Int. J. Bacteriol.* **23**, 256-264.
- Haxo, F. T., Kycia, J. H., Somers, G. F., Bennett, A. & Siegelman, H. W. (1976) *Plant Physiol.* **57**, 297-303.
- Hirs, C. H. W. (1967) *Methods Enzymol.* **11**, 197-199.
- Liu, T.-Y. & Chang, Y. H. (1971) *J. Biol. Chem.* **246**, 2842-2848.
- Olson, J. M. (1971) *Methods Enzymol.* **23**, 636-639.
- Olson, J. M. (1973) *Int. J. Syst. Bacteriol.* **23**, 265-266.
- Olson, J. M. & Romano, C. A. (1962) *Biochim. Biophys. Acta* **59**, 726-728.
- Olson, J. M., Philipson, K. D. & Sauer, K. (1973) *Biochim. Biophys. Acta* **292**, 206-217.
- Olson, J. M., Ke, B. & Thompson, K. H. (1976) *Biochim. Biophys. Acta* **430**, 524-537.
- Pfennig, N. & Biebel, H. (1976) *Arch. Microbiol.* **110**, 3-12.
- Pfennig, N. & Trüper, H. G. (1974) in *Bergey's Manual of Determinative Bacteriology*, 8th edn. (Buchanan, R. E. & Gibbons, N. E., eds.), pp. 24-64, Williams and Williams, Baltimore.
- Rhigetti, P. & Drysdale, J. W. (1971) *Biochim. Biophys. Acta* **236**, 17-28.
- Spackman, D. H., Stein, W. H. & Moore, S. (1958) *Anal. Chem.* **30**, 1190-1206.
- Studier, F. W. (1972) *Science* **176**, 367-376.
- Studier, F. W. (1973) *J. Mol. Biol.* **79**, 237-248.
- Thornber, J. P. & Olson, J. M. (1968) *Biochemistry* **7**, 2242-2250.
- Van Beeumen, J., Ambler, R. P., Meyer, T. E., Kamen, M. D., Olson, J. M. & Shaw, E. K. (1976) *Biochem. J.* **159**, 757-769.