

Donor properties of the three carbonyl groups of chlorophyll *a*: *ab initio* calculations and ^{13}C magnetic resonance studies

(chlorophyll self-aggregation/donor-acceptor interactions/point charge perturbations)

LESTER L. SHIPMAN*, THOMAS R. JANSON*, G. JOSEPH RAY†, AND JOSEPH J. KATZ*

* Chemistry Division, Argonne National Laboratory, Argonne, Illinois 60439; and † Standard Oil Company of Indiana, Naperville, Illinois 60540

Contributed by Joseph J. Katz, June 2, 1975

ABSTRACT The relative donor properties of the three carbonyl groups of chlorophyll *a* have been studied theoretically by a series of *ab initio* molecular fragment, floating spherical Gaussian orbital, self-consistent field calculations on ethyl chlorophyllide *a* and experimentally through a ^{13}C magnetic resonance study on chlorophyll *a*. The approximate ground state electronic wavefunction of ethyl chlorophyllide *a* was perturbed by monopole and dipole point charges whose signs, magnitudes, and positions were chosen to mimic the coulombic interactions associated with carbonyl coordination to Mg. Because the polarizability of the ring V keto carbonyl binding site is substantially greater than that for the ester carbonyl binding sites, the ring V keto binding site binds with smallest binding energy for weak perturbations and with largest binding energy for strong perturbations. A comparison of ^{13}C magnetic resonance chemical shifts in chlorophyll *a* monomer and dimer provides new experimental evidence that the donor-acceptor interactions that bind the chlorophyll dimer together involve a substantial participation by the ring V keto carbonyl and minimal participation by the two ester carbonyl groups, and thus are in agreement with conclusions derived from the *ab initio* calculations.

The primary events (1) in photosynthesis are generally believed to occur within a photosynthetic unit (2-4) in which a large number of chlorophyll molecules act cooperatively. In the photosynthetic unit light energy is absorbed by antenna chlorophyll and converted into electronic excitation energy; this excitation energy is then transferred very efficiently (via excitons) to the photoreactive center (5) where charge separation occurs in a few special chlorophyll molecules (6).

Chlorophyll *a* (Chl *a*) oligomers have been proposed (7) as realistic models for antenna chlorophyll. Infrared (8-10) and proton magnetic resonance studies (11-13) indicate that Chl *a* dimers in nonpolar solvents are probably formed through the coordination of the magnesium of the acceptor Chl *a* molecule to the ring V keto carbonyl oxygen of the donor molecule. ^{13}C magnetic resonance studies (14, 15) have provided direct evidence that the ring V keto carbonyl of the Chl *a* macrocycle participates in coordination to Mg, but the extent, if any, to which the other two ester carbonyl groups participate could not be deduced prior to the present study.

Recently, an *ab initio* self-consistent field (SCF) calculation (16) by the molecular fragment floating spherical Gaussian orbital (FSGO) SCF procedure (17) on ethyl chlorophyllide *a* (Fig. 1) has been successfully carried out. Ethyl chlorophyllide *a* differs from Chl *a* only by the replacement of the phytyl group of Chl *a* by an ethyl group, but is otherwise structurally identical to Chl *a*. This *ab initio* calculation provided, for the first time, an approximate but complete (i.e., both sigma and pi electrons) ground state electronic structure of the ethyl chlorophyllide *a* molecule.

Abbreviations: Chl, chlorophyll; SCF, self-consistent field; FSGO, floating spherical Gaussian orbital; CPU, central processor unit.

In the theoretical part of the present study we have calculated the coulombic binding energies of the three carbonyl functions of Chl *a* (ring V keto carbonyl at position 9, methyl ester or carbomethoxy carbonyl at position 10a, and ethyl ester or propionic carbonyl at position 7c) to gain an understanding of the relative donor abilities of the three carbonyl groups. The *potential* energy component of the free energy of monopole and dipole binding was computed through a series of twelve *ab initio* calculations in which first a monopole and then a dipole was brought up in turn to each of the three carbonyl groups along their C=O axes (Fig. 2) and the binding energy was calculated before and after polarization of the electron distribution.

In the experimental part, we have compared ^{13}C chemical shifts of the carbonyl carbon atoms and of carbon atoms in the vicinity of the potential donor functions in Chl *a* monomer and dimer by a titration procedure introduced by Closs *et al.* (11).

Nuclear geometry of ethyl chlorophyllide *a*

The nuclear geometry for the non-hydrogen atoms used in this study was taken from the x-ray crystallographic study of Strouse (18) with the exception of the terminal carbon atoms of the vinyl group and two ethyl groups (Fig. 1). The terminal C-C bonds of these groups appeared too short in the x-ray study because of large thermal motions perpendicular to the terminal C-C bonds. We lengthened these bonds to 1.39 Å for the vinyl group and 1.53 Å for each of the ethyl groups. Because the hydrogen positions are not well-resolved in the x-ray crystallographic studies of molecules of this size, the hydrogen positions were idealized (D. Spangler, R. McKinney, G. M. Maggiora, L. L. Shipman, and R. E. Christoffersen, to be published).

The *ab initio* molecular fragment FSGO SCF approach

The molecular quantum mechanical approach used in this study was the *ab initio* molecular fragment FSGO SCF approach (17). The ground state wavefunction is a single Slater determinant constructed from doubly occupied molecular orbitals which are linear combinations of floating spherical Gaussian orbitals (FSGO). The positions of the FSGO within the molecular fragments and the FSGO orbital radii were fixed in previous studies (16-18). The linear coefficients for the basis orbitals in each of the molecular orbitals were determined by the usual SCF procedure (20, 21). The approach is *ab initio* in the sense that all integrals are evaluated and none are approximated by empirical data. It has been pointed out (22) that the term "*ab initio*" is sometimes understood to imply greater accuracy than the calculations actually possess. It is, therefore, especially important to dis-

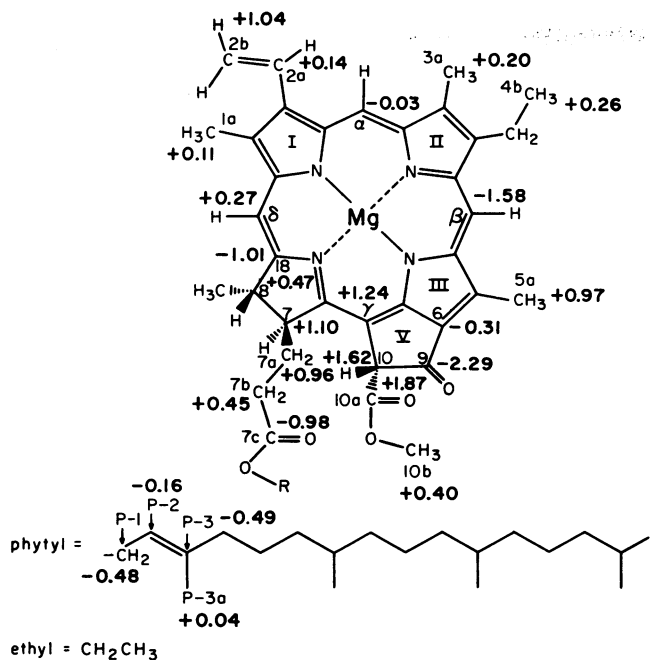


FIG. 1. Structure and partial numbering system for chlorophyll *a* (phytyl) and ethyl chlorophyllide *a* (ethyl). A full numbering system is given in ref. 23. The signed numbers on the structure are the ^{13}C incremental chemical shifts [$\Delta(\text{ppm}) = \delta_{\text{monomer}} - \delta_{\text{dimer}}$] for the indicated carbon atoms which were recorded for Chl *a* enriched to 15% ^{13}C by biosynthesis (25). Spectral data were collected on a Varian CFT-20 spectrometer in pulse Fourier transform mode (3500 pulses, 1 sec recycle time).

cuss the size and quality of the basis set used in our calculations. A total of 275 FSGO contracted to 239 basis functions were used to describe the distribution of the 340 electrons of ethyl chlorophyllide *a*. At 1.4 basis functions per electron pair, this basis must be described as a small basis set. The molecular fragment basis set in its present formulation (17) and without split inner shells (i.e., with one, not two, FSGO to describe each inner shell electron pair) typically gives a total energy of approximately 85% of the energy that would be obtained at the Hartree-Fock limit (i.e., the lowest energy obtainable with a complete basis set and using a single Slater determinant wavefunction). On a chemical scale, the difference between our computed total energy and the exact total energy is large. We compute the total energy of ethyl chlorophyllide *a* to be -1.9×10^3 atomic units (Hartrees), and from this we estimate the Hartree-Fock limit to be $-1.9 \times 10^3 / 0.85 = -2.2 \times 10^3$ atomic units. The difference between our computed total energy and the Hartree-Fock total energy is then 3×10^2 atomic units or 2×10^5 kcal/mol. Approximately two-thirds of this energy difference is associated with the inner shell description because a split inner shell description gives about 95% of the Hartree-Fock limit. The exact total energy lies below the Hartree-Fock limit because electron correlation would further lower the energy; therefore, the difference between our computed total energy and the exact total energy has a lower bound of 1.9×10^5 kcal/mol. This large energy difference is cause for concern, and conclusions drawn from the results of the calculations must be tempered with this in mind. Nevertheless, it has been shown that useful chemical information can be extracted from a wavefunction of this quality. For example, it has been shown (17) that the molecular fragment wavefunction does remarkably well (in spite of the total energy problem characteristic of small basis sets) for such molecular proper-

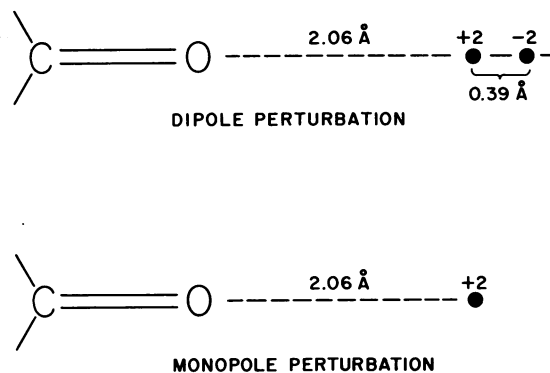


FIG. 2. Point charge magnitudes (in atomic units) and positions for the dipole and monopole perturbations of a carbonyl group used in the *ab initio* calculations.

ties as hydrogen bond energies, equilibrium bond lengths and bond angles, rotational barriers, molecular orbital ordering, and dipole moments. In the present study we are interested in the energy *difference* between the perturbed and unperturbed system, not in the total energies themselves, and we rely upon the assumption that our approximate wavefunction for ethyl chlorophyllide *a* will respond energetically to the coulombic perturbations in much the same way as the exact wavefunction would respond. Further, at the present level of development of large computers and *ab initio* techniques, the molecular fragment procedure used here is a state-of-the-art technique for application to molecules the size of ethyl chlorophyllide *a*.

Computational aspects

All computations were carried out on the IBM 370/195 computer at Argonne National Laboratory. Calculations without polarization (i.e., without SCF iteration to convergence under the influence of the perturbation) required 7 min of central processor unit (CPU)-time while calculations of the monopole and dipole binding energies with polarization (i.e., with SCF iteration to convergence) required an average of 58 and 35 min of CPU-time each, respectively. Each calculation required 610K bytes ($\approx 153\text{K}$ words) of core memory. By way of comparison, the calculation of the unperturbed wavefunction (16) required 2.8 hr of CPU-time, and this time included the one-time calculation of the two-electron integrals. The point charges, which are treated exactly as if they were nuclei, enter into *only* the one-electron nuclear-electron interaction integrals and the nuclear-nuclear interaction terms of the total energy.

Computational procedure

Twelve *ab initio* calculations were performed to evaluate the monopole and dipole coulombic binding energies of the three carbonyl groups of ethyl chlorophyllide *a* (Fig. 1). A dipole was brought up to each of the carbonyl groups in turn and the binding energy calculated before and after SCF iteration to convergence (i.e., after polarization of the electron distribution). The initial charge and bond order matrix was that from the SCF-converged unperturbed ethyl chlorophyllide *a*. This procedure was then repeated for the monopole perturbation. The SCF procedure was taken to be converged when the total energy decreased by less than 0.005 kcal/mol from one iteration to the next. The dipole and monopole used for these calculations are shown in Fig. 2 in their position relative to the carbonyl group. The point charges were chosen to simulate the Mg^{+2} ion for the case of the mono-

Table 1. Monopole and dipole perturbations of the carbonyl groups of ethyl chlorophyllide *a**

	Ring V keto	Methyl ester	Ethyl ester
Monopole perturbation			
Binding energy without polarization	-21.12	-18.93	-29.20
Binding energy with polarization	-68.57	-51.85	-64.05
Dipole perturbation			
Binding energy without polarization	-4.56	-7.27	-7.29
Binding energy with polarization	-6.32	-8.68	-8.64

* All table entries are in units of kcal/mol.

pole perturbation and the dipole formed by the interaction of Mg^{+2} with a macrocycle dianion for the case of the dipole perturbation. The monopole perturbation is expected to be stronger than the real perturbation because the $+2e$ charge on Mg is cancelled by the $-2e$ charge on the macrocycle in the real system. Examination of the electron density maps shows that this cancellation does not occur at distances shorter than the Mg-to-nitrogen lone pair region distance. The magnesium would therefore appear to have a net positive charge to a coordinating group that approaches it to a distance comparable to the Mg-to-nitrogen lone pair distance. Because of this we feel that the strength of the real perturbation in dimerization is closer to that of the monopole perturbation than to the dipole perturbation. If diffuse orbitals reaching out into the region of the point charge perturbation were included in the basis set, it is likely that these diffuse orbitals would be utilized to move electron density onto the positive charge resulting in a lowering of the total energy of the system (A. C. Wahl, personal communication). On the other hand, a real coordination system would have electrons on the perturbant that would tend to repel the electrons in the diffuse orbitals, with the probable result that the diffuse orbitals would not be significantly populated. The distance of the $+2e$ point charge from the carbonyl oxygen (2.06 Å) was taken to be the Mg-to-water oxygen distance found in the x-ray crystal structure of ethyl chlorophyllide *a* (18). The distance between the $+2e$ and $-2e$ charges of the dipole was taken to be the distance (0.39 Å) between the Mg and the plane of the macrocycle nitrogen atoms in this same crystal structure (18); the resulting dipole has a magnitude of 3.7 debyes. Although the absolute values of the binding energies are probably not chemically significant due to the highly approximate simulation of Mg perturbations by point charges, the relative binding energies should serve as a useful indicator of the relative order of binding strengths in the real coordination system.

Results of calculations

The computer binding energies (in kcal/mol) for the monopole and dipole binding to each carbonyl group, both with and without polarization of the electron distribution, are given in Table 1. The binding energy is defined as the energy of the ethyl chlorophyllide *a* plus point charge perturbation (monopole or dipole) minus the energy of the system with the ethyl chlorophyllide *a* and point charge perturbation infinitely separated.

The order of the absolute binding energies for monopole binding *without polarization* is ethyl ester $C=O >$ ring V

keto $C=O >$ methyl ester $C=O$, and the corresponding order for dipole binding is ethyl ester $C=O \approx$ methyl ester $C=O >$ ring V keto $C=O$. Thus, we see that the binding energy is sensitive to the nature of the perturbing coulombic potential and also that the ethyl ester $C=O$ is consistently the site of greatest binding energy (although the methyl ester carbonyl binding site binds the dipole with an essentially equal binding energy).

The order of the absolute binding energies for monopole binding *with polarization* of the electron distribution is ring V keto $C=O >$ ethyl ester $C=O >$ methyl ester $C=O$, and the corresponding order for dipole binding is methyl ester $C=O \approx$ ethyl ester $C=O >$ ring V keto $C=O$. Thus, for monopole binding, the ring V keto $C=O$ binding site is clearly the site of greatest binding energy. On the other hand, the ester carbonyl binding sites have greater binding energies than the ring V keto $C=O$ for dipole binding.

The polarization energy is defined as the binding energy with polarization of the electronic distribution minus the binding energy without polarization. For monopole binding, the polarization energies are -47.45, -32.92, and -34.85 kcal/mol for the ring V keto $C=O$, methyl ester $C=O$, and ethyl ester $C=O$ binding sites, respectively, while the polarization energies for dipole binding to these sites are -1.76, -1.41, and -1.35 kcal/mol, respectively. Thus, for both monopole and dipole binding, the ring V keto $C=O$ binding site is clearly the most polarizable of the three. In the case of monopole binding, the polarization energy accounts for a significant fraction of the total binding energy. Specifically, the polarization energy is 69, 63, and 54% of the total binding energy for the ring V keto, methyl ester, and ethyl ester carbonyl binding sites, respectively. It is important to note that because of the difference in polarization energy between the ring V keto and ester carbonyl binding sites, the former is the site of smallest binding energy without polarization and the site of greatest binding energy for the strong monopole perturbation with polarization. The greater polarization energy for the ring V keto carbonyl binding site is a direct result of the participation of the keto carbonyl group in a 28-electron π -system within which there is general charge redistribution upon coordination. The two ester carbonyl groups each participate in π -systems containing only 4 electrons.

Experimental procedure

The chemical shifts of all 55 carbon atoms in monomeric Chl *a* have now been assigned (15, 23). In the present study, the ^{13}C chemical shifts of chlorophyll *a* dimer were assigned by a titration procedure in which $[^2H_8]$ tetrahydrofuran was added step-wise to a dry 0.1 M $[^2H_6]$ benzene solution until the dimer was fully disaggregated to monomer. The incremental changes in the chemical shifts recorded during the course of the titration permit assignment of the ^{13}C chemical shifts in the dimer.

Experimental results

The differences in the ^{13}C chemical shifts (for the indicated carbon atoms) of the dimer relative to the monomer are shown in Fig. 1, with positive and negative values indicating shifts to higher and lower field, respectively. The chemical shift differences in the dimer relative to the monomer are the resultant of (a) a ring current contribution from the adjacent macrocycle that can be positive or negative depending upon the location of the influenced carbon atom (24); (b) general electron redistribution effects from the donor-acceptor

tor interactions, which in the case of a participating C=O group gives rise to a large (downfield) deshielding; and (c) conformational changes. Because we are yet unable to isolate the electron redistribution and conformational components of the incremental shifts for carbon atoms (other than those in C=O groups) that are a part of the conjugation pathway of the Chl *a* macrocycle (e.g., carbon atoms C₆, C₁₈, and C_β), we confine the present discussion largely to *sp*³ carbon atoms not on the conjugation pathway and to the C₉, C_{7c}, and C_{10a} carbon atoms in the carbonyl groups.

The incremental chemical shift for the carbon atom in a ¹³C=O coordinated to Mg is the sum of a large, necessarily positive, incremental shift from the ring current effect and a large, necessarily negative, incremental shift from the coordination electron redistribution effect. The incremental shift of a neighboring *sp*³ ¹³C atom should (to a first approximation) result from the large positive shift from the ring current effect. Our criteria for a coordinated ¹³C=O therefore are: (a) neighboring *sp*³ ¹³C atoms must have large positive incremental shifts; and (b) the ¹³C=O carbon atom must at the same time show an incremental shift significantly more negative than any of the nearby *sp*³ ¹³C atoms. According to our criteria and the data of Fig. 1, only ¹³C₉=O of the three C=O groups in Chl *a* shows evidence for substantial coordination to Mg. (a) The ring V keto carbonyl group: the incremental shift of ¹³C₉ is the most negative of any carbon atom and is 3.91 ppm more negative than the incremental shift of ¹³C₁₀. The incremental shift of the neighboring ¹³C₁₀ is positive. (b) The methyl ester carbonyl group: the ¹³C_{10a}=O group does not appear to coordinate to a significant extent because its incremental shift is positive relative to the incremental chemical shifts of the neighboring ¹³C₁₀ and ¹³C_{10b}. (c) The phytol ester carbonyl group: for ¹³C₇=O the situation is more complicated but is also interpreted to indicate minimal donor participation. Carbon atoms 7, 7a, and 7b in the propionic acid side chain show decreasing positive incremental shifts, which become negative at carbon 7c, and continue with diminishing negative values through the first three carbon atoms of the phytol chain. The incremental shift of C_{7c} is only 0.50 ppm more negative than carbon P1. The order of these incremental shifts is consistent with a ring current origin [the methylene protons at 7a and 7b show a positive ¹H ring current shift (8)], with the isoshielding surface that divides the shielding and deshielding zones of the adjoining macrocycle ring current passing between carbon atoms 7b and 7c. In addition, the ¹³C_{7c} resonance in the dimer spectrum is very sharp (23), strongly suggesting that it enjoys free motion and does not participate to a significant extent as a donor in the dimer.

Summary

The molecular fragment FSGO SCF approach provides useful qualitative information about the properties of a molecule even as large as chlorophyll. Our theoretical and experimental results, taken together, indicate that (i) for weak perturbations, such as hydrogen bonding, the binding energies of the three carbonyl groups are quite similar in magnitude;

and (ii) under the strong electron withdrawing perturbation associated with coordination to Mg, the ring V keto carbonyl binding site is a site of substantially greater binding energy than either of the two ester carbonyl binding sites. The theoretical results indicate that the greater binding energy for the ring V keto carbonyl binding site is due primarily to the greater polarizability of that binding site region and is not due to any inherent charge buildup in that region of the unperturbed molecule.

This work was performed under the auspices of the Energy Research and Development Administration. We thank Dr. A. C. Wahl for many helpful discussions and guidance in regard to the calculations in this study.

1. For a recent review see, Bearden, A. J. & Malkin, R. (1975) *Quart. Rev. Biophys.* **7**, 131-177.
2. Emerson, R. & Arnold, W. (1931-1932) *J. Gen. Physiol.* **15**, 391-420.
3. Emerson, R. & Arnold, W. (1932-1933) *J. Gen. Physiol.* **16**, 191-205.
4. Clayton, R. K. (1971) *Adv. Chem. Phys.* **19**, 353-378.
5. Clayton, R. K. (1973) *Annu. Rev. Biophys. Bioeng.* **2**, 131-156.
6. Katz, J. J. & Norris, J. R. (1973) in *Current Topics in Bioenergetics* (Academic Press, New York), Vol. 5, pp. 41-75.
7. Cotton, T. M., Trifunac, A. D., Ballschmitter, K. & Katz, J. J. (1974) *Biochim. Biophys. Acta* **368**, 181-198.
8. Katz, J. J., Closs, G. L., Pennington, F. C., Thomas, M. R. & Strain, H. H. (1963) *J. Am. Chem. Soc.* **85**, 3801-3809.
9. Anderson, A. F. H. & Calvin, M. (1964) *Arch. Biochem. Biophys.* **107**, 251-259.
10. Henry, M. & Leicknam, J.-P. (1970) *Colloq. Int. Cent. Nat. Rech. Sci.* **191**, 317-333.
11. Closs, G. L., Katz, J. J., Pennington, F. C., Thomas, M. R. & Strain, H. H. (1963) *J. Am. Chem. Soc.* **85**, 3809-3821.
12. Pennington, F. C., Strain, H. H., Svec, W. A. & Katz, J. J. (1964) *J. Am. Chem. Soc.* **86**, 1418-1426.
13. Katz, J. J., Dougherty, R. C. & Boucher, L. J. (1966) in *The Chlorophylls*, eds., Vernon, L. P. & Seely, G. R. (Academic Press, New York), chap. 7, pp. 185-251.
14. Katz, J. J., Janson, T. R., Kostka, A. G., Uphaus, R. A. & Closs, G. L. (1972) *J. Am. Chem. Soc.* **94**, 2883-2885.
15. Katz, J. J. & Janson, T. R. (1973) *Ann. N.Y. Acad. Sci.* **206**, 579-603.
16. Spangler, D., McKinney, R., Maggiora, G. M., Shipman, L. L. & Christoffersen, R. E. (1975) *Chem. Phys. Lett.*, in press.
17. Christoffersen, R. E., Spangler, D., Maggiora, G. M. & Hall, G. G. (1973) *J. Am. Chem. Soc.* **95**, 8526-8536.
18. Strouse, C. E. (1973) *Proc. Nat. Acad. Sci. USA* **71**, 325-328.
19. Christoffersen, R. E. (1972) *Adv. Quantum Chem.* **6**, 333-393.
20. Hall, G. G. (1951) *Proc. Roy. Soc. London Ser. A* **205**, 541-552.
21. Roothaan, C. C. J. (1951) *Rev. Mod. Phys.* **23**, 69-89.
22. Bingham, R. C., Dewar, M. J. S. & Lo, D. H. (1975) *J. Am. Chem. Soc.* **97**, 1285-1293.
23. Boxer, S. G., Closs, G. L. & Katz, J. J. (1974) *J. Am. Chem. Soc.* **96**, 7058-7066.
24. Johnson, C. E. & Bovey, F. A. (1958) *J. Chem. Phys.* **29**, 1012-1014.
25. Flaumenhaft, E., Uphaus, R. A. & Katz, J. J. (1970) *Biochim. Biophys. Acta* **215**, 421-429.