Minimization of Polypeptide Energy, I. Preliminary Structures of Bovine Pancreatic Ribonuclease S-Peptide

Kenneth D. Gibson, Harold A. Scheraga

Proceedings of the National Academy of Sciences of the United States of America, Volume 58, Issue 2 (Aug. 15, 1967), 420-427.

*MINIMIZATION OF POLYPEPTIDE ENERGY, I. PRELIMINARY STRUCTURES OF BOVINE PANCREATIC RIBONUCLEASE S-PEPTIDE**

By Kenneth D. Gibson and Harold A. Scheraga†

DEPARTMENT OF CHEMISTRY, CORNELL UNIVERSITY, ITHACA, NEW YORK

Communicated June 20, 1967

Because the problem of computing protein structures from a knowledge of the amino acid sequence is so complex, we have approached it by making many simplifying assumptions and then removing these assumptions in stages until a computer program could ultimately be developed to yield the structure of a protein. Thus, using a hard-sphere potential, it was possible to compute the allowed conformations of dipeptides and tripeptides,[1] of helical structures,[2, 3] of a cyclic octapeptide,[4] and of the cyclic decapeptide gramicidin-S.[5] Subsequently, the hard-sphere potential was replaced by more complete energy expressions, and energy contours were computed for several dipeptides[6, 7] and helical structures.[6, 8–10] Similar calculations have been reported by Ramachandran,[11, 12] Liquori,[13, 14] and Flory.[15, 16]

In this paper, we make certain simplifying assumptions about the energies, and introduce the effect of the solvent (i.e., water). In addition, we report briefly on the exploration of several minimization techniques, and apply the most satisfactory one to the 20-residue N-terminus (S-peptide) of bovine pancreatic ribonuclease. Because of the problem of multiple apparent local minima, the structures reported here are to be regarded only as preliminary ones. In subsequent papers, we will report on energy minimization for some small polypeptides containing closed loops, and also on another minimization procedure which permits rapid minimization, starting with many arbitrary initial conformations.

Calculation of the Energy.—Although we have shown that variations in bond lengths and bond angles affect the energy contours of dipeptides,[7] we have not yet found it necessary to include this feature in calculations for small polypeptides; hence, the backbone and side-chain geometry were held fixed in the calculations reported in this paper. As before,[9] a Lennard-Jones pairwise 6-12 potential function was used for the nonbonded interactions. However, in order to reduce the amount of computation, hydrogen atoms were not considered individually unless they can take part in a strong hydrogen bond. Instead, they were regarded as part of an extended "atom" such as a methylene group, etc. The coefficients of the attractive terms in the potentials were calculated by means of the Slater-Kirkwood equation,[17] using the values of α and N_{eff} given in Table 1. The coefficients of the repulsive terms were adjusted so as to minimize the pair-interaction energy when the interatomic distance was equal to the sum of the van der Waals radii as given in reference 15 or reference 20, *plus* 0.2 Å (see ref. 16). This is equivalent to enlarging the van der Waals radii; the values of the enlarged radii are given in Table 1.

Electrostatic interactions were computed by assigning partial charges to each atom and summing the contributions of all pairs, using Coulomb's law.[9, 21] The partial charges were essentially those given elsewhere.[21] Zero charge was assigned

TABLE 1

DATA FOR EVALUATING NONBONDED AND SOLVENT ENERGIES

Atom type	α (Polarizability)* ($cm^3 \times 10^{24}$)	(N_{eff})†	van der Waals radius‡ (Å)	A (No. of solvent molecules in first shell)	$F°$ (Free energy for removing one solvent molecule) (kcal/mole)
H	0.42	0.9	1.30	2	0.31
O(carbonyl)	0.84	6	1.60	4	0.94
O(hydroxyl)	0.59	6	1.60	6	0.84
O^-(carboxyl)	2.14	6	1.60	5	4.80
N(amide)	1.15	6	1.65	2	0.63
NH_3^+(amine)	2.13	9	1.75	5	15.40
N^+(imidazole)	2.03	6	1.65	3	3.30
N^+(guanidine)	2.03	6	1.65	6	1.20
CH(aliphatic)	1.35	6	1.95	2	−0.13
CH_2(aliphatic)	1.77	7	1.95	3	−0.13
CH_3(aliphatic)	2.17	8	1.95	8	−0.13
C(aromatic)	1.65	5	1.80	2	0.11
CH(aromatic)	2.07	6	1.90	3	0.11
S	0.34	16	1.90	6	−0.17

* Taken from refs. 15, 18, and 19, or estimated from data given in these references.
† Estimated from ref. 17, Fig. 1.
‡ Values from refs. 15 and 20, augmented by 0.1 Å.

to most nonpolar atoms. The dielectric constant was taken as 3.0 to reflect the fact that the polypeptides are assumed to be in aqueous solution.

Allowance was made for hydrogen bonds by adding an orientation-dependent attractive term to the nonbonded potential for every pair of atoms that can form such a bond. The form of the total nonbonded potential (excluding electrostatic terms) is

$$U_{NB} = \frac{a}{r^{12}} - \frac{b}{r^6} - \frac{U_H}{r^6} f(\theta). \tag{1}$$

The coefficient U_H of the attractive term was calculated by summing all the remaining electrostatic and nonbonded energy contributions of the pair of atoms involved in the bond, and requiring that the distance between the hydrogen atom and the acceptor atom be 1.85 Å when U_{NB} is a minimum (with $f(\theta)$ set equal to 1). This procedure appeared to give reasonable values for U_{NB} at the minimum; however, it is difficult to make a valid comparison with experimental results, since the latter always include many interactions between atoms not taken into account in the calculation of U_H. Angular dependence was introduced through the function $f(\theta)$, where

$$f(\theta) = \begin{cases} 0 \text{ for } 0° \leq \theta \leq 135° \\ \cos^4 2\theta \text{ for } 135° < \theta \leq 180° \end{cases} \tag{2}$$

and θ is the angle between the vectors A-H and $B \ldots$H, with A being the donor atom and B the acceptor atom. For a linear hydrogen bond, $\theta = 180°$. This function is equal to 1.0 when the bond is linear and falls off rapidly to zero as the bond departs from linearity. No account was taken of possible angular dependence involving the orientation around the acceptor atom.

The hydrogen bond and 6-12 potentials become negligible at interatomic distances of more than a few angstroms. The electrostatic energies fall off more slowly with distance; but since a sufficiently large separation of the atoms allows solvent molecules (in this case water, with a dielectric constant of about 80) to

come between them, we believe that it is reasonable to neglect the electrostatic contribution when the atoms are not close together. However, simply neglecting the interactions between all pairs of atoms separated by more than a certain distance gives rise to very small discontinuities which cause insuperable problems in the minimization procedure. Therefore, each of the above types of pairwise interaction energies was multiplied by the factor

$$g(r) = \begin{cases} \dfrac{\left(1 - \dfrac{r^2}{r_0^2}\right)^4}{0.0001\left(\dfrac{r}{r_0}\right)^8 + \left(1 - \dfrac{r^2}{r_0^2}\right)^4} & \text{for } 0 \leq r < r_0. \\ 0 & \text{for } r \geqq r_0 \end{cases} \tag{3}$$

This function is very nearly equal to 1.0 when $r < 0.9r_0$, then drops to a value of 0.5 when $r = 0.95r_0$, and to zero when $r = r_0$; furthermore, it is continuous and has a continuous derivative. The value of r_0 was taken as the sum of the van der Waals radii given in Table 1 *plus* 2.0 Å.

The nonbonded, electrostatic, and hydrogen-bond energies were computed for a pair of atoms only if they are separated by more than one bond about which rotation can occur; for example, the interaction between the backbone (amide) H and O atoms of the same residue were calculated in this way, but not those between the H and C′ atoms or between the N and O atoms. Instead, the contributions of atom pairs that are closer together are included in the torsional potentials. (This treatment of the torsional terms differs from that in refs. 8 and 9 because hydrogen atoms are treated differently in the present paper.) In fact, experimental values for torsional potentials include such atomic interactions as well as an intrinsic bond potential;[17] we have, therefore, taken rotational barriers for side chains from experimental data wherever there was a close analogy with a known compound (see Table 2). For the backbone rotations, where there is probably a low intrinsic barrier and most of the restriction to rotation arises from nonbonded interactions,[6] the torsional potential was calculated by the method given in reference 17; the same procedure was used for bond 4 of the arginine side chain. The intrinsic bond potential was taken as 0.5 kcal/mole for all these bonds.

In contrast to previous computations,[1–16] a new feature in the present work is the inclusion of a free-energy term to describe the influence of the solvent, i.e., water. The following considerations provided the basis for obtaining an expression for this contribution. Calculations of the thermodynamic properties of aqueous solutions of nonpolar solutes[25] and of alkali halide ions[26] show that the nearest-neighbor solvent molecules in the first shell around the solute molecule contribute very much more than all other solvent molecules to the free energy of solvation of these substances; the same is probably true of polar nonionic solutes. Hence, unless two atoms approach each other to within a distance equal to the sum of their van der Waals radii plus the diameter of a water molecule, the solvent that is displaced is assumed not to contribute to the energy. Also, as soon as an atom has approached another atom within this distance, it will displace a certain amount of solvent, which should be roughly proportional to the volume of the displacing atom; further approach of the two atoms, up to their van der Waals distance, should not

TABLE 2

SIDE-CHAIN ROTATIONAL BARRIERS[a]

Bond no.[b]	Residue	χ_{trans}, deg	χ_{gauche}, deg	ΔU (trans→gauche) (kcal/mole)	Barrier height[c] (kcal/mole)
1[d]	Aromatic	300	60, 180	0.40	3.50[e]
1[d]	Branched	180, 300	60	0.40	3.80
1[d]	Serine	300	60, 180	0.20	1.00
1[d]	Other	300	60, 180	0.20	3.50[e]
2	Leucine, isoleucine	180, 300	60	0.75	3.50[e]
2	Aromatic	—	—	—	0.00[f]
2	Serine	60, 180, 300	—	—	2.00[g]
2	Asparagine	60, 180, 300	—	—	0.50[h]
2	Aspartate	—	—	—	0.00[f]
2	Other	180	60, 300	0.75	3.50[i]
3	Methionine	180	60, 300	0.40	2.00[j]

[a] Barriers not shown in this table were the same as for some analogous bond in the table; e.g., bond 3 of glutamine is analogous to bond 2 of asparagine, bond 3 of lysine or arginine is analogous to bond 2 of lysine, etc.
[b] See ref. 22 for conventions.
[c] Barrier height relative to the trans position.
[d] Assignment of trans and gauche minima and choice of ΔU for bond 1 was based on data of Pachler.[23]
[e] Analogous to iso-butane.[24]
[f] Sixfold potential considered negligible.
[g] Analogous to ethanol.[24]
[h] Analogous to acetic acid.[24]
[i] Analogous to propane.[24]
[j] Estimate.

greatly increase the amount of solvent displaced. To describe this behavior, we have expressed the amount of water q_{ij} removed from nearest-neighbor contact with the ith atom by the approach of the jth atom as

$$q_{ij} = V_j g(r_{ij}). \tag{4}$$

Here r_{ij} is the distance between the atoms, $g(r)$ is the function given in equation (3), and V_j is a factor proportional to the volume of the jth atom. The total amount of solvent removed from the ith atom by the approach of all other atoms will then be

$$W_i = \sum_{j \neq i} q_{ij}. \tag{5}$$

In practice, the values of V_j were set equal to the volumes of the atoms (in Å^3), as given in reference 20, divided by 30. A further consideration in computing the solvation energy is that there is a maximum solvation number for any atom; when this number of solvent molecules has been removed, there can be no further contribution to the energy from the removal of solvent molecules from the first shell around that atom. If the maximum solvation number of the ith atom is A_i, then the free energy contribution arising from the removal of solvent from this atom is

$$F_i = \sum_i F_i^\circ \phi(W_i, A_i). \tag{6}$$

F_i° is the free-energy change when one solvent molecule is removed from the ith atom and $\phi(W_i, A_i)$ is a function which is equal to zero when $W_i = 0$, to A_i when $W_i \geq A_i$, and is continuous in between. The total solvent free energy is then obtained by summing over all atoms:

$$F_W = \sum_i F_i. \tag{7}$$

The choice of ϕ used in this work was

$$\phi(W,A) = A\left[1 - \exp\left(-\frac{W}{A - W}\right)\right]. \quad (8)$$

This is nearly linear up to $W = 0.75A$, then turns sharply but smoothly to a constant value of A. A simpler function, consisting of two polynomials pieced together, has also been used, with essentially the same results. A more realistic form for ϕ would have several steps, to reflect the fact that the solvent is removed discontinuously; an appropriate function is now being investigated. Values for A_i were deduced with the aid of Corey-Pauling-Koltun space-filling models; those of F_i° were computed from reference 25 for nonpolar groups and were calculated for ionic groups by the method in reference 26. For nonionized polar groups, approximate values were deduced from thermodynamic data for organic compounds containing polar groups.[27] The values of A_i and F_i° are included in Table 1. It should be emphasized that the F_i°'s, and hence also F_W, are *free* energies. Our experience to date shows that the most pronounced effect of the solvent free energy is to cause charged ionic groups to stick out from the surface of the polypeptide into the water; we would expect that, in larger structures, the solvation contribution will also cause the nonpolar groups to lie preferentially in the interior of the macromolecule.

Energy Minimization.—The energy is expressed as a function of *all* the dihedral angles of the backbone and side chains, and then minimized by allowing for a continuous variation of all of the dihedral angles. The ultimate aim of this approach is to locate the global minimum of the energy. At present the only known way to achieve this result is to try to find all local minima and choose the one with the lowest energy. However, we are also investigating a method for locating the global minimum in which searches for local minima alternate with searches for any conformation of lower energy.

In this paper, we report results obtained with one of the many methods for finding local minima that have been reported in the literature. Our choice was based on an evaluation of seven such methods, during the course of which we found that one method was outstandingly more effective than any of the others. The methods investigated were: steepest descents, conjugate gradients,[28] Davidon's variable metric method[29] (all of which require the computation of gradients as well as energies), Rosenbrock's method,[30] Powell's method,[31] Smith's method,[32] and a version of the simplex method [33] (none of which requires gradients). Initially, the methods were tested with hexa-L-alanine, which is an 11-variable problem, according to the approach described in the previous section. Based on computational efficiency, this test served to eliminate the first two and the last two methods. With larger polypeptides, such as hypertensin (38 variables), it was found that, with both Rosenbrock's and Powell's methods, in any iteration only about one third of the directions chosen for line searches led to a significant drop in the energy or alteration of the structure. As the number of variables was increased, the proportion of line searches leading to a significant alteration of structure decreased even further. Thus, these two methods became less and less efficient for higher-dimensional problems. Only Davidon's method performed satisfactorily, with any number of variables up to 110 (a 31-residue peptide from lysozyme), the largest number which we have tried so far. In our hands, this procedure has always led to a

stationary point in fewer than $3n$ line searches, where n is the number of independent variables. The results reported here were obtained with Davidon's procedure.

Results and Discussion.—Five different conformations of ribonuclease S-peptide were used as starting points for energy minimization. The first was an approximate α-helix with $\phi = 120°$, $\psi = 130°$,[22] and the second an approximate β conformation with $\phi = 60°$, $\psi = 300°$. The third conformation had $\phi = 100°$, $\psi = 150°$, and the fourth had $\phi = 60°$, $\psi = 180°$; these do not correspond to any known stable helix. In the fifth conformation, the first ten residues were set in the α-helical conformation ($\phi = 120°$, $\psi = 130°$) and the remaining ten in the β conformation ($\phi = 60°$, $\psi = 300°$). All ionizable groups with the exception of the histidine and tyrosine side chains were taken to be charged in all cases. Minimization was continued until every component of the gradient was less than 0.05 kcal/mole/radian in magnitude.

TABLE 3

BACKBONE DIHEDRAL ANGLES OF FIVE MINIMUM-ENERGY CONFORMATIONS OF S-PEPTIDE

Starting point for minimization: Final structure:*	α-Helix		β Conformation		$\phi = 100°$, $\psi = 150°$		$\phi = 60°$, $\psi = 180°$		α-Helix and β conformation	
	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ
1. Lysine	—	263.5	—	339.3	—	291.6	—	325.2	—	314.4
2. Glutamate	83.1	150.9	51.1	264.9	88.1	164.3	92.7	262.2	88.1	143.4
3. Threonine	85.0	160.9	77.5	307.2	89.0	181.9	87.5	133.7	113.8	151.4
4. Alanine	98.5	125.6	79.1	332.4	98.6	138.9	90.7	262.7	88.9	150.1
5. Alanine	121.2	149.3	78.7	331.9	90.3	171.9	70.1	214.5	117.4	126.2
6. Alanine	114.5	126.3	78.0	325.6	88.3	153.7	77.3	224.1	120.4	139.7
7. Lysine	126.3	134.5	84.2	314.8	115.8	149.5	74.8	210.8	124.8	120.7
8. Phenylalanine	124.9	121.7	70.5	322.8	104.0	140.8	38.8	213.1	128.1	125.3
9. Glutamate	132.8	125.3	126.4	302.6	74.1	217.2	42.1	211.5	138.3	118.1
10. Arginine	133.7	118.9	86.3	307.3	77.2	154.3	57.0	311.3	132.9	123.1
11. Glutamine	134.9	125.0	55.0	320.6	116.1	151.0	76.3	198.0	121.2	291.6
12. Histidine	135.4	124.3	101.8	290.1	94.3	157.0	33.3	252.3	103.2	296.3
13. Methionine	134.9	119.4	52.6	257.5	124.8	146.6	84.9	257.6	89.6	309.6
14. Aspartate	134.2	124.7	92.1	310.8	100.5	131.2	82.5	251.2	127.2	305.1
15. Serine	138.2	123.3	87.8	358.3	114.6	172.7	47.3	124.4	63.3	315.0
16. Serine	132.5	126.0	77.1	321.4	87.6	132.6	80.1	205.5	89.5	317.5
17. Threonine	133.9	122.6	58.1	303.6	117.3	134.6	84.5	150.2	58.7	303.6
18. Serine	133.8	123.3	28.8	303.0	86.2	222.8	88.5	188.9	28.8	302.8
19. Alanine	136.3	123.9	83.8	317.2	89.9	146.0	38.0	215.9	63.2	318.1
20. Alanine	116.3	139.4	34.4	296.4	37.5	122.8	53.5	139.9	31.6	300.9
Final energy (kcal/mole)	48.82		−19.97		−5.39		19.84		−17.16	

* For clarity, only backbone dihedral angles are listed.

The results clearly indicated the presence of five apparent local minima, each rather close to the starting point (Table 3). In each case, at most six dihedral angles changed by more than 60°, and many of the angles did not change by more than 20°. The final conformations showed the same sort of regularity as the initial ones. Thus, the structure that was reached from the α-helical starting point was an irregular helix with approximately the same pitch and the same hydrogen bonding as an α-helix. Similarly, starting at the β conformation led to an irregular β conformation as the final structure. The third, fourth, and fifth structures also showed approximately the same regularity as their respective starting points. At present, we cannot say definitely that any of the five final conformations represents a true local minimum of our energy expression, since the second derivatives of this

expression have not been computed; and without this information it is impossible to be sure that a stationary point located by Davidon's method is a true minimum.

The implications of these results are clear enough, even though they are not entirely unexpected. The earlier studies of helical homopolymers[9, 10] showed that the α-helix and β conformation are both local minima. Our results suggest that when the restrictions of homogeneity and regularity are removed, the peptide can still find irregular minimal conformations close to the original regular ones. Thus, the presence of many different side chains, as in the S-peptide, need not by itself provide enough of a perturbation to overcome the cooperative interactions that occur in a regular structure. A further implication, at least for small peptides, is that there may be many dissimilar minima with almost the same energy (cf. the second and fifth conformations in Table 3); this was also apparent in the studies of helical homopolymers.[9, 10] It remains to be seen how far these conclusions can be carried over to proteins. In later papers, we will report on the effect of the presence of closed loops, which provide more drastic constraints on the peptide chain, and also on another minimization procedure which permits rapid minimization starting with *many* arbitrary initial conformations.

Our thanks are due to Nancy Moxley and Mark Thompson for their valuable programing assistance. Nearly all the computations described here were carried out on the CDC-6600 computer at the AEC Computing Center at New York University. The remainder were performed on the CDC-1604 computer at Cornell University.

* This work was supported by a research grant (GB-4766) from the National Science Foundation, and by a research grant (GM-14312) from the National Institute of General Medical Sciences of the National Institutes of Health, U.S. Public Health Service.

† To whom requests for reprints should be addressed.

[1] Leach, S. J., G. Nemethy, and H. A. Scheraga, *Biopolymers*, **4**, 369 (1966).

[2] *Ibid.*, **4**, 887 (1966).

[3] Nemethy, G., D. C. Phillips, S. J. Leach, and H. A. Scheraga, *Nature*, **214**, 363 (1967).

[4] Nemethy, G., and H. A. Scheraga, *Biopolymers*, **3**, 155 (1965).

[5] Scheraga, H. A., S. J. Leach, R. A. Scott, and G. Nemethy, *Discussions Faraday Soc.*, **40**, 268 (1965).

[6] Scott, R. A., and H. A. Scheraga, *J. Chem. Phys.*, **45**, 2091 (1966).

[7] Gibson, K. D., and H. A. Scheraga, *Biopolymers*, **4**, 709 (1966).

[8] Ooi, T., R. A. Scott, G. Vanderkooi, R. F. Epand, and H. A. Scheraga, *J. Am. Chem. Soc.*, **88**, 5680 (1966).

[9] Ooi, T., R. A. Scott, G. Vanderkooi, and H. A. Scheraga, *J. Chem. Phys.*, in press.

[10] Scheraga, H. A., R. A. Scott, G. Vanderkooi, S. J. Leach, K. D. Gibson, T. Ooi, and G. Nemethy, *Conformation of Biopolymers*, ed. G. N. Ramachandran (New York: Academic Press, in press).

[11] Ramachandran, G. N., C. Ramakrishnan, and V. Sasisekharan, *J. Mol. Biol.*, **7**, 95 (1963).

[12] Ramachandran, G. N., C. M. Venkatachalam, and S. Krimm, *Biophys. J.*, **6**, 849 (1966).

[13] de Santis, P., E. Giglio, A. M. Liquori, and A. Ripamonti, *J. Polymer Sci.*, **A1**, 1383 (1963).

[14] Liquori, A. M., *J. Polymer Sci.*, **C12**, 209 (1966).

[15] Brant, D. A., and P. J. Flory, *J. Am. Chem. Soc.*, **87**, 663, 2791 (1965).

[16] Brant, D. A., W. G. Miller, and P. J. Flory, *J. Mol. Biol.*, **23**, 47 (1967).

[17] Scott, R. A., and H. A. Scheraga, *J. Chem. Phys.*, **42**, 2209 (1965).

[18] Ketelaar, J., *Chemical Constitution* (Amsterdam: Elsevier Pub. Co., 1953), p. 91.

[19] Kondratyev, V., *The Structure of Atoms and Molecules* (Groningen, Netherlands: Noordhof, 1964), p. 429.

[20] Bondi, A., *J. Phys. Chem.*, **68**, 441 (1964).

[21] Poland, D., and H. A. Scheraga, *J. Phys. Chem.*, submitted.

[22] Edsall, J. T., P. J. Flory, J. C. Kendrew, A. M. Liquori, G. Nemethy, G. N. Ramachandran, and H. A. Scheraga, *Biopolymers*, **4**, 121 (1966); *J. Biol. Chem.*, **241**, 1004 (1966); *J. Mol. Biol.*, **15**, 399 (1966).

[23] Pachler, K. G. R., *Spectrochim. Acta*, **20**, 581 (1964).

[24] Wilson, E. B., Jr., *Advan. Chem. Phys.*, **2**, 367 (1959); Lin, C. C., and J. D. Swaley, *Rev. Mod. Phys.*, **31**, 841 (1959); Green, J. H. S., *Quart. Rev. London*, **15**, 125 (1961).

[25] Nemethy, G., and H. A. Scheraga, *J. Chem. Phys.*, **36**, 3401 (1962).

[26] Griffith, J. H., and H. A. Scheraga, *J. Chem. Phys.*, submitted.

[27] Frank, H. S., and M. W. Evans, *J. Chem. Phys.*, **13**, 507 (1945).

[28] Fletcher, R., and C. M. Reeves, *Computer J.*, **7**, 149 (1964).

[29] Davidon, W. C., AEC Research and Development Report, ANL-5990 (1959); R. Fletcher and M. J. D. Powell, *Computer J.*, **6**, 163 (1963).

[30] Rosenbrock, H. H., *Computer J.*, **3**, 175 (1961); R. Fletcher, *Computer J.*, **8**, 33 (1965).

[31] Powell, M. J. D., *Computer J.*, **7**, 303 (1965).

[32] Smith, C. S., NCB Scientific Dept. Report no. SC846/MR/40 (1962).

[33] Nelder, J. A., and R. Mead, *Computer J.*, **7**, 308 (1965).