Volume 3 no.6 June 1976 Nucleic Acids Research

Ring current shielding effects in nucleic acid double helices

David B. Arter and Paul G. Schmidt.

University of Illinois, School of Chemical Sciences, Urbana, IL 61801, USA

Received 19 February 1976

ABSTRACT

Values of ring current shielding in parts-per-million have been calculated for double helical nucleic acids in the A-RNA (RNA-11), A'-RNA (RNA-12) and B-DNA geometries. Atomic coordinates determined previously from x-ray diffraction of fibers were used to calculate the positions of protons relative to both nearest and second nearest neighboring bases, including those on the complementary strand. The magnitude of the diamagnetic shielding was then calculated for each aromatic ring. From these calculations tables were constructed for use in determining the shielding expected for carbon-bound and ring nitrogen-bound protons of any double helical nucleic acid sequence. The results are compared with available experimental data for several oligonucleotides and with previous ring current shielding calculations where differences of up to 0.4 ppm are found.

INTRODUCTION

A substantial share of the recent interest in nuclear magnetic resonance spectra of oligoribo- and deoxyribonucleotides has been focussed on chemical shift effects of the ring currents of the aromatic bases.[1] A quantitative theory of the origin of this effect has been developed by Waugh and Fessenden[2] and Johnson and Bovey.[3] More recently, Geissner-Prettre and Pullman[4-6] calculated the magnitudes of the ring currents in a variety of aromatic ring systems of biological interest and from these computed the ring current shielding exerted by the four common bases, adenine, guanine, cytosine and uracil. They published their results as a series of isoshielding contours situated in a plane parallel to and 3.4 angstroms above (or below) the plane of the base. These contours have been the basis of most previous calculations of ring current shielding for double helical oligonucleotides. Recently Borer, Kan and Ts'o[7] have used accurate atomic models of A'-RNA and B-DNA to measure distances of protons relative to the planes of the bases. Because of the pronounced "tilt" and "twist" of bases in an RNA double helix the planes of adjacent bases are not parallel. With the aid of isoshielding contours for distances other than 3.4 angstroms, Borer, *et al*., determined ring current induced shifts for protons of double

© Information Retrieval Limited 1 Falconberg Court London W1V 5FG England

helical ApApGpCpUpU , with which our results are in substantial agreement.

In this paper we present the results of a series of calculations which represent the present limits of accuracy for the determination of ring current shielding in double helical oligonucleotides.

METHODS

The calculation of the shielding in parts-per-million was done using the same equation used by Giessner-Prettre and Pullman[6] to calculate the iso-shielding contours. Elliptical integrals were calculated using CEL1 and CEL2 from the Fortran Scientific Subroutine Library. These subroutines were able to reproduce published tables of complete elliptical integrals over the entire range of the modulus, k, from 0 to 0.9998. As a further check, we reproduced portions of the isoshielding contours at 3.4Å from the base planes. Our results were identical to those of Geissner-Prettre and Pullman.[6]

The coordinates used in our calculations were based on the x-ray data of Arnott, et. al.[8,9] To obtain the coordinates of a given proton with respect to a given 5- or 6-membered ring in a base, we first constructed a coordinate transformation to translate the origin of the dyadic axis system to the center of the ring. We then rotated the translated system so that the new positive x-axis passed through N3 of a purine 6-membered ring or N1 of a pyrimidine ring and the new positive y-axis passed through the midpoint of the C4-C5 bond of a purine 6-membered ring or the midpoint of the C5-C6 bond of a pyrimidine ring. The new z axis was made perpendicular to both x and y. The angles between the new axes were found to be $90^{0} \pm 0.2^{0}$, and the coordinates of the shielding base were found to be in the x-y plane of the new axis system to within 0.01Å in every case. To obtain the ring-centered axis system for the purine 5-membered rings, that for the 6-membered ring was simply translated to the center of the 5-membered ring.

The coordinates of the protons themselves were not available from the x-ray data, but were computed as follows. The base protons were assumed to lie in the plane of the base along a line joining the atom to which they are attached to the atom on the opposite side of the ring (or, in the case of H8, to the midpoint of the C4-C5 bond). Carbon-proton distances were taken to be 1.08Å and nitrogen-proton distances to be 1.01Å. For the ribose H1' we assumed a C-H bond length of 1.07Å and tetrahedral geometry about C1', then used the Hydrogen Position Program of the Syntex STL computer system to calculate the coordinates.

The calculations described above were performed separately for each of the four bases in each of the three geometries, A-RNA (RNA-11), A'-RNA (RNA-12) and B-DNA. This served as a valuable internal check on the compu-

tations, since the coordinates of the protons on adenosine (or uracil) are almost identical to those of the protons on guanosine (or cytosine). The results showed that the shielding of a given proton on one purine (or pyrimidine) differed from that of the same proton on the other purine (or pyrimidine) by a maximum of 0.02 ppm. In fact this difference was observed in only two cases; a difference of 0.01 ppm was observed in 14 cases and no difference was observed in the remaining 128 cases. Copies of the FORTRAN Computer Program are available from the authors on request.

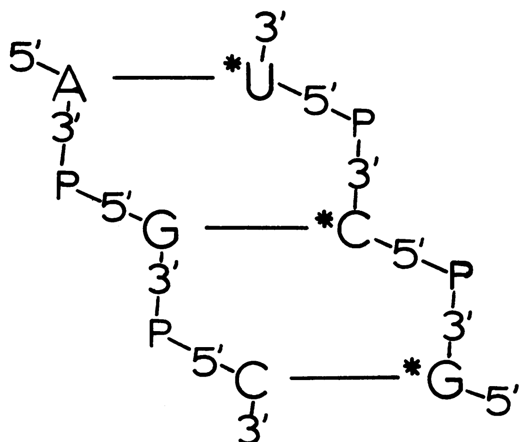

Figure 1. The ApGpC strand is the principle strand so the A-*U pair is above the G-*C pair. Shielding of the protons on the G-*C pair by A-*U is first order from above. Shielding of the protons on the A-*U pair by C-*G is second order from below.

RESULTS AND DISCUSSION

Results of the calculations are presented in Tables I-IV. Our conventions are as follows and are illustrated in Fig. 1. In a given double helix one strand is chosen to be the principle strand and the other is the complementary strand. Bases or atoms in the complementary strand are identified by an asterisk. Of course, in practice it makes no difference which strand is chosen as the principle strand; the distinction is entirely artificial. On the principle strand, a base is above a given base if it is on the 5' side, and below if it is on the 3' side of the given base. The opposite holds on the complementary strand. First order shielding refers to shielding by a nearest neighbor, second order to shielding by a second nearest neighbor. Cross strand shielding refers specifically to shielding of the carbon-bound protons on a given base by the base to which it is hydrogen bonded. As expected these values are independent of the helix geometry.

Tables I-III give the shielding of each proton by an entire base pair from above and below. Note that H1' is listed separately since its coordinates are independent of the base. To illustrate their use consider the trimer in Figure 1. We will determine the shielding for each proton in this helix for the A-RNA geometry. The protons in the A-*U pair are R-H1', Pu-H8 and A-H2 on the A and *R-H1, *Py-H6, *Py-H5 and *U-NH on the *U. There is no shielding from above. First order shielding from below is by G-*C; second order shielding from below is by C-*G. For the G-*C pair, there is no second order shielding. First order shielding from above is by A-*U and from below is by C-*G. For the C-*G pair, first and second order shieldings from above are by G-*C and A-*U, respectively. The results are summarized in Table V.

One novel result of these calculations is the appearance of negative values of the shielding. While these rarely exceed -.01 to -.02 ppm, in certain cases they can be as high as -.06 ppm and, in the case of cross strand effects, -.08 ppm for the H-5 of uracil. Moreover, as illustrated in Table V, it is not unusual for these effects to add to give a net deshielding, particularly for the ribose H1' protons and for the base protons on a terminal base pair.

A more significant finding is that of substantial differences between predictions of 1st order shielding made by our calculations for the protons in RNA double helices and those which have appeared previously in the literature. For example, in Table VI we compare our results for the NH protons to estimates published by Shulman, et al.[10] and Kearns and Wong[11] for A'-RNA and by Patel and Tonelli[12] for A'-RNA and B-DNA. Patel and Tonelli have also computed ring current parameters for the non-exchangeable protons in A-RNA and B-DNA.[12] Our results are in good agreement with the earlier shielding values for both sets of protons in B-DNA but show marked differences for the protons in the RNA helices. In one case (Shulman, et al.[10]) at least part of the differences are due to the fact that these authors increased their computed values by an additional 20% to better fit the available experimental data. In general, however, it seems that the chief source of disagreement is the apparent difficulty of accurately adjusting shielding values taken from the 3.4 Å isoshielding contours to account for the large variations from 3.4 Å of the perpendicular distance of the various protons from the planes of adjacent bases. We have found, for example, that in A-RNA the actual distances can vary from 2.5 Å to 3.9 Å for the carbon-bound protons and from 3.2 Å to 3.6 Å for the NH protons.

Table VII summarizes the data available to date on the carbon-bound

Table I. Shielding in the A-RNA Helix

of	by AU	by UA	by GC	by CG	by AU	by UA	by GC	by CG
	First Order from Above				First Order from Below			
R-H1'	0.01	0.05	0	0.01	0.03	-.01	0.03	0.01
*R-H'	-.01	0.03	0.01	0.03	0.05	0.01	0.01	0
Pu-H8	0.62	0.04	0.36	0.12	-.06	-.03	-.03	-.02
A-H2	0.06	0.65	0.11	0.35	0.97	0.06	0.59	0.21
G-NH	0.09	0.73	0.17	0.42	0.87	0.14	0.41	0.15
*Py-H6	-.03	-.06	-.02	-.03	0.03	0.46	0.08	0.27
*Py-H5	-.03	-.06	-.03	-.04	0.14	1.47	0.42	1.02
*U-NH	0.06	0.54	0.19	0.36	0.61	0.30	0.27	0.16
Py-H6	0.46	0.03	0.27	0.08	-.06	-.03	-.03	-.02
Py-H5	1.47	0.14	1.02	0.42	-.06	-.03	-.04	-.03
U-NH	0.30	0.61	0.16	0.27	0.54	0.06	0.36	0.19
*Pu-H8	-.03	-.06	-.02	-.03	0.04	0.62	0.12	0.36
*A-H2	0.06	0.97	0.21	0.59	0.65	0.06	0.35	0.11
*G-NH	0.14	0.87	0.15	0.41	0.73	0.09	0.42	0.17
	Second Order from Above				Second Order from Below			
R-H1'	0.04	0.11	0.03	0.06	0	0	0	0
*R-H1'	0	0	0	0	0.11	0.04	0.06	0.03
Pu-H8	0.12	0.10	0.07	0.06	-.01	0	-.01	0
A-H2	0.03	0.09	0.03	0.06	0.08	0.02	0.06	0.03
G-NH	0.04	0.08	0.03	0.05	0.11	0.05	0.07	0.05
*Py-H6	0	-.01	0	-.01	0.10	0.10	0.05	0.06
*Py-H5	0	-.01	0	-.01	0.09	0.22	0.08	0.13
*U-NH	0.03	0.05	0.03	0.03	0.13	0.07	0.08	0.06
Py-H6	0.10	0.10	0.06	0.05	-.01	0	-.01	0
Py-H5	0.22	0.09	0.13	0.08	-.01	0	-.01	0
U-NH	0.07	0.13	0.06	0.08	0.05	0.03	0.03	0.03
*Pu-H8	0	-.01	0	-.01	0.10	0.12	0.06	0.07
*A-H2	0.02	0.08	0.03	0.06	0.09	0.03	0.06	0.03
*G-NH	0.05	0.11	0.05	0.07	0.08	0.04	0.05	0.03

Table II. Shielding in the A'-RNA Helix

of	by AU	by UA	by GC	by CG	by AU	by UA	by GC	by CG
	First Order from Above				First Order from Below			
R-H1'	-.02	0.04	-.02	0	0.02	-.01	0.02	0
*R-H1'	-.01	0.02	0	0.02	0.04	-.02	0	-.02
Pu-H8	0.71	0.04	0.40	0.14	-.05	-.03	-.03	-.02
A-H2	0.05	0.68	0.12	0.37	1.03	0.06	0.64	0.23
G-NH	0.10	0.80	0.20	0.46	0.97	0.13	0.46	0.16
*Py-H6	-.03	-.05	-.02	-.03	0.03	0.51	0.09	0.30
*Py-H5	-.03	-.05	-.02	-.03	0.14	1.50	0.42	1.01
*U-NH	0.07	0.59	0.21	0.41	0.68	0.27	0.29	0.15
Py-H6	0.51	0.03	0.30	0.09	-.05	-.03	-.03	-.02
Py-H5	1.50	0.14	1.01	0.42	-.05	-.03	-.03	-.02
U-NH	0.27	0.68	0.15	0.29	0.59	0.07	0.41	0.21
*Pu-H8	-.03	-.05	-.02	-.03	0.04	0.71	0.14	0.40
*A-H2	0.06	1.03	0.23	0.64	0.68	0.05	0.37	0.12
*G-NH	0.13	0.97	0.16	0.46	0.80	0.10	0.46	0.20
	Second Order from Above				Second Order from Below			
R-H1'	0.03	0.12	0.03	0.07	0	0	0	0
*R-H1'	0	0	0	0	0.12	0.03	0.07	0.03
Pu-H8	0.13	0.11	0.07	0.06	-.01	0	-.01	0
A-H2	0.03	0.09	0.04	0.06	0.09	0.02	0.06	0.03
G-NH	0.03	0.08	0.03	0.05	0.12	0.04	0.08	0.05
*Py-H6	0	-.01	0	-.01	0.11	0.11	0.06	0.06
*Py-H5	0	-.01	0	0	0.11	0.22	0.07	0.12
*U-NH	0.03	0.05	0.03	0.04	0.14	0.06	0.09	0.05
Py-H6	0.11	0.11	0.06	0.06	-.01	0	-.01	0
Py-H5	0.22	0.11	0.12	0.07	-.01	0	0	0
U-NH	0.06	0.14	0.05	0.09	0.05	0.03	0.04	0.03
*Pu-H8	0	-.01	0	-.01	0.11	0.13	0.06	0.07
*A-H2	0.02	0.09	0.03	0.06	0.09	0.03	0.06	0.04
*G-NH	0.04	0.12	0.05	0.08	0.08	0.03	0.05	0.03

Table III. Shielding in the B-DNA Helix

of	by AU	by UA	by GC	by CG	by AU	by UA	by GC	by CG
	First Order from Above				First Order from Below			
R-H1'	0	-.01	-.01	-.01	0.16	0.01	0.17	0.08
*R-H1'	0.01	0.16	0.08	0.17	-.01	0	-.01	-.01
Pu-H8	0.15	-.01	0.09	0.02	-.01	-.02	0	0
A-H2	0.04	0.78	0.04	0.31	1.00	0.04	0.46	0.10
G-NH	0.21	0.89	0.14	0.38	0.68	0.41	0.27	0.18
*Py-H6	-.02	-.01	0	0	-.02	0.08	0	0.06
*Py-H5	-.02	-.01	-.01	0	0.06	0.64	0.20	0.49
*U-NH	0.11	0.83	0.15	0.40	0.32	0.78	0.15	0.32
Py-H6	0.08	-.02	0.06	0	-.01	-.02	0	0
Py-H5	0.64	0.06	0.49	0.20	-.01	-.02	0	-.01
U-NH	0.78	0.32	0.32	0.15	0.83	0.11	0.40	0.15
*Pu-H8	-.02	-.01	0	0	-.01	0.15	0.02	0.09
*A-H2	0.04	1.00	0.10	0.46	0.78	0.04	0.31	0.04
*G-NH	0.41	0.68	0.18	0.27	0.89	0.21	0.38	0.14
	Second Order from Above				Second Order from Below			
R-H1'	0.02	0.06	0.02	0.03	0.05	0.01	0.03	0.02
*R-H1'	0.01	0.05	0.02	0.03	0.06	0.02	0.03	0.02
Pu-H8	0.05	0.03	0.03	0.02	0.02	0.01	0.01	0.02
A-H2	0.06	0.23	0.07	0.13	0.20	0.06	0.12	0.07
G-NH	0.10	0.18	0.07	0.10	0.19	0.12	0.11	0.08
*Py-H6	0.01	0.02	0.02	0.01	0.03	0.04	0.02	0.02
*Py-H5	0.03	0.02	0.03	0.02	0.04	0.12	0.03	0.06
*U-NH	0.09	0.15	0.07	0.09	0.18	0.13	0.09	0.08
Py-H6	0.04	0.03	0.02	0.02	0.02	0.01	0.01	0.02
Py-H5	0.12	0.04	0.06	0.03	0.02	0.03	0.02	0.03
U-NH	0.13	0.18	0.08	0.09	0.15	0.09	0.09	0.07
*Pu-H8	0.01	0.02	0.02	0.01	0.03	0.05	0.02	0.03
*A-H2	0.06	0.20	0.07	0.12	0.23	0.06	0.13	0.07
*G-NH	0.12	0.19	0.08	0.11	0.18	0.10	0.10	0.07

Table IV. Cross-Strand Shielding

Proton	Shielding
A-H1'	0
A-H8	0
A-H2	-.02
G-H1'	-.01
G-H8	-.01
C-H1'	-.04
C-H6	-.03
C-H5	-.04
U-H1'	-.07
U-H6	-.05
U-H5	-.08

Table V. Summary of the Shielding in (ApGpC)·(GpCpU), A-RNA

base pair	proton	2nd order from above	1st order from above	Cross-Strand	1st order from below	2nd order from below	Total
	R-H1'	--	--	0	0.03	0	0.03
	A-H8	--	--	0	-.03	0	-.03
	A-H2	--	--	-.02	0.59	0.03	0.60
A-*U	*R-H1'	--	--	-.07	0.01	0.03	-.03
	*U-H6	--	--	-.05	0.08	0.06	0.09
	*U-H5	--	--	-.08	0.42	0.13	0.47
	*U-NH	--	--	--	0.27	0.06	0.33
	R-H1'	--	0.01	-.01	0.01	--	0.01
	G-H8	--	0.62	-.01	-.02	--	0.59
G-*C	G-NH	--	0.09	--	0.15	--	0.24
	*R-H1'	--	-.01	-.03	0	--	-.04
	*C-H6	--	-.03	-.03	0.27	--	0.21
	*C-H5	--	-.03	-.04	1.02	--	0.95
	R-H1'	0.04	0	-.03	--	--	0.01
	C-H6	0.10	0.27	-.03	--	--	0.34
C-*G	C-H5	0.22	1.02	-.04	--	--	1.20
	*R-H1'	0	0.01	-.01	--	--	0
	*G-H8	0	-.02	-.01	--	--	-.03
	*G-NH	0.05	0.15	--	--	--	0.20

Table VI. Comparison of First Order Shielding Effects for the Exchangeable NH Protons.

5′ 1 —— 3 3′
(G / T,U) — (C / A)
3′ 2 —— 4 5′

		GC							TA or UA						
		I	II	III	IV	V	VI	VII	I	II	III	VI	V	VI	VII
1	3														
C	G	.46	.7	.7	.42	.7	.38	.3	.29	.6	.6	.27	.3	.15	.2
G	C	.20	.25	.25	.17	.25	.14	.2	.15	.1	.1	.16	.2	.32	.35
T,U	A	.80	1.2	1.0	.73	1.3	.89	.8	.68	1.3	1.1	.61	.75	.32	.3
A	T,U	.10	.1	.1	.09	.1	.21	.25	.27	0	0	.30	.35	.78	.9
2	4														
C	G	.16	.2	.2	.15	.15	.18	.25	.21	.2	.2	.19	.3	.15	.15
G	C	.46	.7	.7	.41	.45	.27	.25	.41	.6	.6	.36	.7	.40	.35
T,U	A	.13	.1	.1	.14	.2	.41	.5	.07	.1	.1	.06	.1	.11	.15
A	T,U	.97	1.3	1.1	.87	1.05	.68	.5	.59	.7	.6	.54	1.1	.83	.8

I A'-RNA, this paper.
II A'-RNA, after Shulman, *et al.*[10]
III A'-RNA, after Kearns and Wong.[11]
IV A-RNA, this paper.
V A-RNA, after Patel and Tonelli.[12]
VI B-DNA, this paper.
VII B-DNA, after Patel and Tonelli.[12]

Table VII. Comparison of Predicted and Observed Shielding for Various Oligoribonucleotides.

	Proton(s)	$\Delta\delta_{obs}$	$\Delta\delta_{calc}$	
A.	poly (A) · poly (U)[a]		A-RNA	A'-RNA
	U-H6	.01	0.05	0.06
	A-H8	.86	0.58	0.78
	A-H2	1.09	1.12	1.18
B.	ApApGpCpUpUp[b]		A'-RNA	B-DNA
	A_1-H1'	.27	.02	0.19
	A_1-H8	.22	-.06	0
	A_1-H2	1.36	1.09	1.10
	A_2-H1'	.69	0	0.19
	A_2-H8	.67	.68	0.17
	A_2-H2	.76	.70	0.55
	G_3-H1'	.35	0	0.10
	G_3-H8	.93	.81	0.20
	C_4-H1'	.50	-.04	-.01
	C_4-H_6	.48	.35	0.06
	C_4-H5	.97	1.15	0.57
	U_5-H1'	.38	-.05	-.05
	U_5-H6	.07	.07	-.05
	U_5-H5	.51	.43	0.16
	U_6-H1'	.12	.04	-.05
	U_6-H6	.07	.04	-.05
	U_6-H5	.25	.13	.01
C.	CpCpGpG[c]		A'-RNA	B-DNA
	C_1-H6	0.15	-.06	-.02
	C_2-H6	0.03	0.02	-.02
	G_3-H8	0.58	0.17	0.03
	G_4-H8	0.76	0.45	0.10

[a]from Heller, *et al.*[13] The assignments are ours and are not confirmed.
[b]from Borer, *et al.*[7]
[c]unpublished data from Arter and Schmidt. Chemical shifts were measured at 360 MHz at ~1.0 mM strand concentration in 1 M NaCl at 4°C.

protons of ribonucleic acid double helices along with the calculated shielding values. The experimental values are obtained by subtracting from the shifts of the monomers at low temperature (extrapolated to infinite dilution) those of the corresponding protons in the helix at low temperature. The most significant data are those obtained by Heller, et al.,[13] for the poly(A) · poly(U) copolymer since the x-ray data upon which our coordinates are based were obtained from fibers of this copolymer. Our calculations predict the positions of the carbon bound protons in the A-RNA geometry to be 7.97 (H-6), 7.93 (H-8) and 7.10 (H-2); and in the A'-RNA geometry 7.96 (H-6), 7.73 (H-8) and 7.04 (H-2) in parts-per-million from 2,2-dimethylsilapentane-5-sulfonate (DSS). The observed spectrum showed a resonance at 7.13 ppm and a broad, less well defined region of intensity with maxima at approximately 7.6 and 8.0 ppm. Since at the salt concentration used to measure these shifts the A-RNA geometry is favored,[14] our calculations apparently are able to predict the positions of the H-2 and H-6 resonances with a high degree of accuracy, but are less successful in the case of the H-8 resonances.

As can be seen from Table VII, predicted values are also reasonably close to the observed values for base protons of the A-U rich A_2GCU_2 helix.[7] The largest discrepancies are found for the terminal residues but we feel that these are within the range to be expected if there is end-to-end aggregation of the helices. Our calculations shed no light on the unusual behavior of the H1' protons which are probably influenced most strongly by something other than ring current shielding. Among the possibilities are local anisotropic effects and ribose ring strain induced by the close stacking of the helix.

Poor agreement is found for the guanosine H8 resonances of C_2G_2. We believe this is primarily due to the tendency of G rich oligonucleotides to aggregate extensively in solution in other than an end-to-end manner.

ACKNOWLEDGEMENTS

We wish to thank Professor Iain Paul for his assistance in computing the coordinates of the H1' protons. This work was supported by the National Institutes of Health grant GM 18038. DBA is a National Institutes of Health Predoctoral Trainee.

REFERENCES

1 Ts'o, P. O. P. (1974) Basic Principles in Nucleic Acid Chemistry, Vols. I, II, New York, N. Y. Academic Press.
2 Waugh, J. S. and Fessenden, R. W. (1957) J. Amer. Chem. Soc. 79 846-849.
3 Johnson, C. E. and Bovey, F. A. (1958) J. Chem. Phys. 29, 1012-1014.

4 Geissner-Prettre, C. and Pullman, B. (1965) C. r. hebd. Seanc. Acad. Sci. Paris 261, 2521-2523.

5 Geissner-Prettre, C. and Pullman, B. (1969) C. r. hebd. Seanc. Acad. Sci. Paris 268, 1115-1117.

6 Geissner-Prettre, C. and Pullman, B. (1970) J. Theor. Biol. 27, 87-95.

7 Borer, P. N., Kan, L. S. and Ts'o, P. O. P. (1975) Biochemistry 14, 4847-4863.

8 Arnott, S. and Hukins, D. W. L. (1972) Biochem. Biophys. Res. Commun. 47, 1504-1509.

9 Arnott, S., Hukins, D. W. L. and Dover, S. D. (1972) Biochem. Biophys. Res. Commun. 48, 1392-1399.

10 Shulman, R. G., Hilbers, C. W., Kearns, D. R., Reid, B. R. and Wong, Y. P. (1973) J. Mol. Biol. 78, 57-70.

11 Kearns, D. R. and Wong, Y. P. (1974) J. Mol. Biol. 87, 755-774.

12 Patel, D. J. and Tonelli, A. E. (1975) Biochemistry 14, 3990-3996.

13 Heller, M. J., Tu, A. T. and Maciel, G. E. (1974) Biochemistry 13, 1623-1631.

14 Arnott, S., Fuller, W. Hodgson, A. and Prutton, I. (1968) Nature 220 561-567.