



Consultant Progress Report
IICA/EMBRAPA-PROCENSUL II
BIOPHYSICS AND MOLECULAR BIOLOGY

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BIOPHYSICS AND MOLECULAR BIOLOGY

**Consultant Progress Report
IICA/EMBRAPA-PROCENSUL II**

Goran Neshich

Brasilia, agosto de 1989

**INSTITUTO INTERAMERICANO DE COOPERAÇÃO PARA A AGRICULTURA
EMPRESA BRASILEIRA DE PESQUISA AGROPECUARIA**

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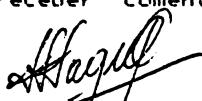
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As atividades de consultoria são realizadas no âmbito do Projeto de Desenvolvimento da Pesquisa Agropecuária e Difusão de Tecnologia na Região Centro-Sul do Brasil - FPOCENSUL II, financiado parcialmente pelo Banco Interamericano de Desenvolvimento - BID e a EMBRAPA conforme os contratos de Empréstimo 139/IC-BR e 760/SF-BR, assinados em 14 de março de 1985 entre o Governo Brasileiro e o BID.

As opiniões dos consultores são inteiramente pessoais e não refletem, necessariamente, o ponto de vista do IICA ou da EMBRAPA.

A coordenação dos Contratos IICA/EMBRAPA agradeceria receber comentários sobre estes relatórios.



Horacio H. Stagno
Coordenador Contratos IICA/EMBRAPA



INTER-AMERICAN INSTITUTE FOR COOPERATION ON AGRICULTURE
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CONSULTANT PROGRESS REPORT
(FIRST SEMESTER)

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2. Specialist in: Biophysics and Molecular Biology
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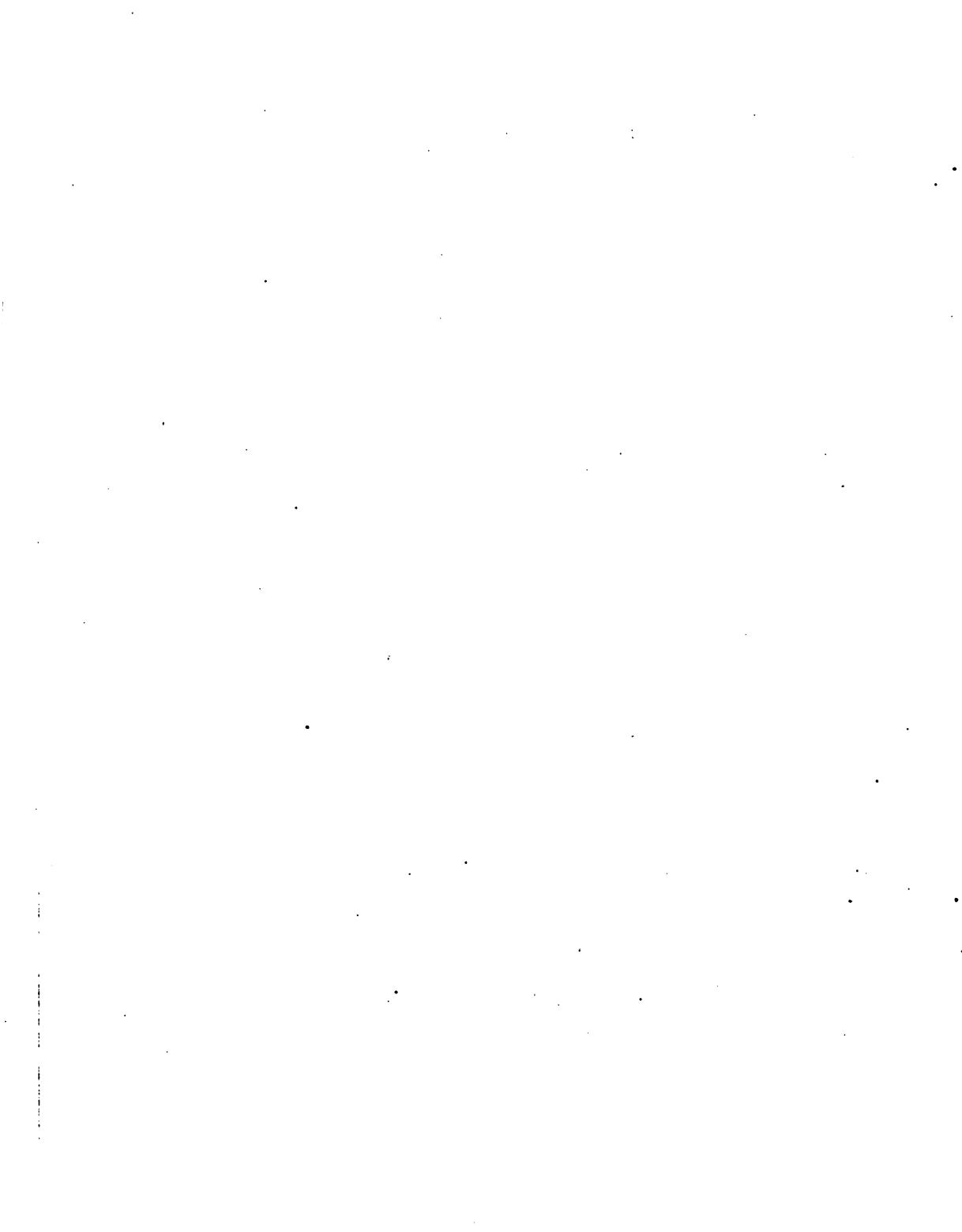
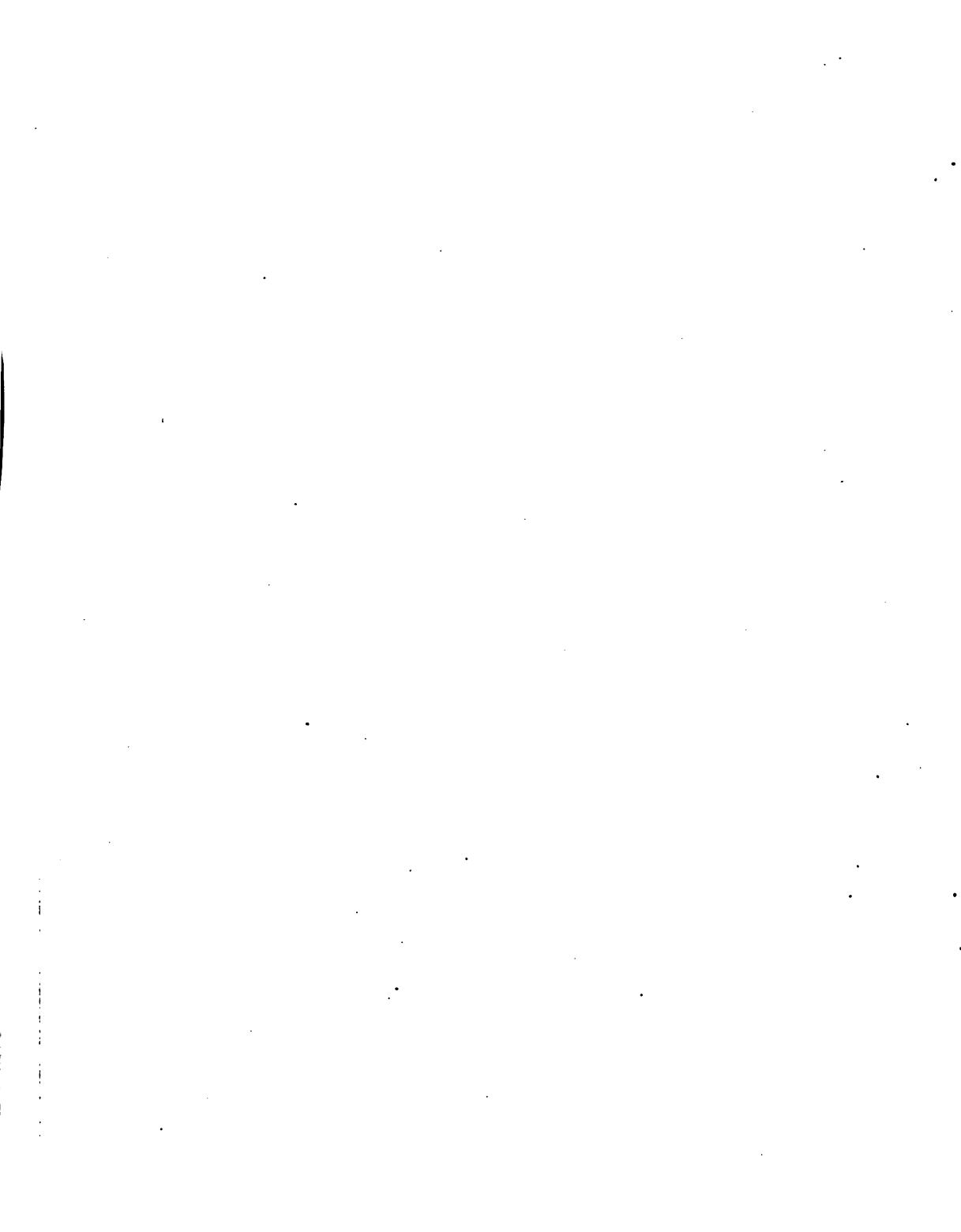


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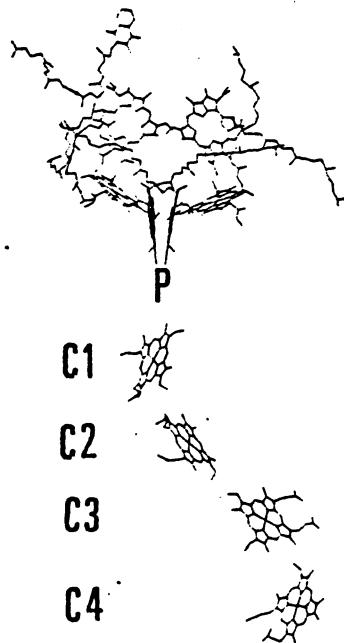
1) Introduction:

During this period major emphasis has been done on the quantum-mechanical calculation of the electron transfer probability for the process of the electron sharing between two high potential hemes C1 and C2 (see fig. 1 on the following page) in the cytochrome C subunit of the RC protein of the photosynthetic bacterium *Rhodopseudomonas viridis*. These calculations are of ample importance for deciphering the role of each heme (and therefore of respective neighboring protein milieu) in the mechanism of electron transfer and energy transduction in primary processes of photosynthesis. Our calculations have indicated nature of low-potential heme interplay between the two high-potential hemes, details of which are explained in the last version of our scientific paper (to be presented on the VIII international congress on Photosynthesis in Stockholm -Sweden August 5 to August 13. 1989.)

Results presented are starting point for molecular genetics experiments to be designed for critical estimation of the sites to be modified in the primary sequence of the protein. Mutant should be specifically altered (in the functional sense, also) and this alteration should be predictable by appropriate biophysical analysis. Further research emphasis will be along this line.

Included in this report is the preliminary study of the secondary structure of the replicase protein "foot and mouth

disease virus", project in development with the UFRJ department of biophysics. Our primary goal here is to define the active site specific structure and eventually predict appropriate changes to block it (probably sterically). Further work is currently being done and will be described in the next trimestral report.



2)

TEMPERATURE DEPENDENCE OF THE RATE OF ELECTRON TRANSFER BETWEEN
 HIGH POTENTIAL HEMES IN C SUBUNIT AND SPECIAL PAIR OF
 REACTION CENTER PROTEIN OF PHOTOSYNTHETIC BACTERIA
RHODOPSEUDOMONAS VIRIDIS

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Abstract:

The temperature dependence of high-potential cytochrome photooxidation in *Rhodopseudomonas viridis* is presented. Two reactions, (I) electron transfer from C1' to P' (fast), and (II) electron transfer from C3 to C1 (slow) (where P' is the primary donor and C1 and C3 are the first and third cytochromes in line from P') produce biphasic kinetics. The rates of both reactions decrease with decreasing temperature down to about 200K and become almost insensitive to temperature below. The implications for electron transfer theory are discussed.

KEY WORDS:

Rhodopseudomonas viridis; Electron transfer; Temperature dependence

1. INTRODUCTION

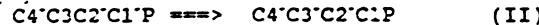
The bacterium *Rhodopseudomonas viridis* has four c-type cytochromes integrated into a single protein subunit of the reaction center complex. According to x-ray analysis of the crystallized reaction center complex [1] the cytochromes, C1, C2, C3, C4, are arranged approximately linearly in this order extending from P. Two of

ABBREVIATIONS: BCHl2, P940. P, the bacteriochlorophyll special pair which serves as the primary electron donor in the photo-induced charge separation; C1, C2, C3, C4, secondary cytochromes in order with C1 closest to BCHl2. This is different from the numbering in [1]; DAD, 2,3,5,6-tetraethyl-p-phenylenediamine; THPPD, N,N,N',N'-tetramethyl-p-phenylenediamine; MOPS, 4-morpholinolcarboxylic acid; ET, electron transfer.

them have high redox midpoint potentials (cyt-c559, $E_m = 380$ mV and cyt-c556, $E_m = 310$ mV). The other two have low midpoint potential (cyt-c552), $E_m = 0$ mV and cyt-c554, $E_m = -60$ mV). Several lines of evidences [2,3] assign the two high-potential hemes to C1 (c559) and C3 (c556), and the two low-potential hemes to C2 and C4. These cytochromes interact with P/P' whose $E_m = 500$ mV. We have measured the temperature dependence of the rate of oxidation of high potential cytochromes following absorption of a short pulse of light from a ruby laser. At redox potential, E , about 200 to 250 mV both high potential cytochromes are reduced and thus capable of donation an electron to the oxidized primary donor, while the low-potential cytochromes are already oxidized. Under these conditions the absorbance change observed at 554 nm is characterized by an initial jump due to photo-oxidation of P and reduction of acceptor Q_a. This is followed by a fast exponential phase due to oxidation of C1 by P':



and then a slow exponential phase due to transfer of an electron from C3 to C1' [3]:



This paper will present the results of measurements of the temperature dependence of the rates of these reactions [4].

2. MATERIAL AND METHODS

Reaction centers from *R. viridis* were prepared by a modification [5,6] of the procedure described in [7]. In the experiments they were used at a concentration of 20 μM in a buffer of 10 mM MOPS, 100 mM NaCl, 10 mM K-pyrophosphate, pH 9. For use at low temperature 30% glycerol was added.

For all of the experiments secondary acceptor quinone, Q_a, was absent from reaction centers. Nevertheless, any possible remaining secondary acceptor activity was inhibited by 4 mM o-phenanthroline. Redox mediators used were DAD and TMPD, present at total concentrations of 25 μM and 20 μM , respectively.

To keep the redox potential of the sample between 200 and 250 mV, 50 μM ascorbate was present.

All kinetic experiments were performed on the laser spectrophotometer described in [8]. A split-beam arrangement was used to monitor both the measuring light entering and than leaving the sample cuvette. The latter was suspended in an Oxford Instruments CF204 cryostat cooled by a stream of nitrogen gas pumped from liquid nitrogen. A beam-splitter combined both the measuring light and the laser beam to the same path through the cryostat. The path-length through the sample was 1.3 mm. Data were digitized and analyzed by computer.

3. RESULTS

The kinetics observed at 554 nm (initial jump plus two exponential phases) continued with increasing half-times for both phases as the temperature was lowered until the point was reached

at which the rates of both phases became approximately constant with further lowering of temperature. The results are summarized in Table 1 and Fig. 1. The "transition temperature" refers to the point of intersection of asymptotes to the temperature dependent and temperature independent parts as shown in Fig. 1.

Shopes et al. [3], observing at 945 nm, showed that all the flash induced P' recovers in a single kinetic phase with a half-time of 187 nsec at room temperature. This correlates with the rate observed for reaction (I) and corroborates that P' is involved in reaction (I) and not in reaction (II).

The points plotted in Fig. 1 are, usually, averages of 9 individual traces already compensated for the measuring light fluctuation by the split beam technique.

We found that the peak at 555 nm, observable at room temperature in the spectrum of the fast phase did not shift significantly as the temperature was lowered (data not shown).

Further data on the amplitudes of the different phases is not yet completely analyzed and will be presented elsewhere. For now we mention that the amplitudes of the exponential phases did not change much, if at all, with decreasing temperature.

4. DISCUSSION

We see that the oxidation of the two high-potential cytochromes in *Rhodopseudomonas viridis* have the same type of temperature profile (rate decreasing from room temperature to around 100-200K, then becoming near temperature-independent for all lower temperatures) as has been observed in *Chromatium vinosum*, both high- (Sarai and DeVault, unpublished) and low-potential [9] cytochromes, in *Rhodopseudomonas gelatinosa* and *Rhodopseudomonas sp. NW* [10], but not in *Ectothiorhodospira shaposhnikovii* [11]. Most of the above species are known to have four cytochromes bound to the reaction center and thus, presumably, a structure similar to that of *Rhodopseudomonas viridis* [1].

The present data have several implications for electron transfer theory:

(1) The single frequency version of non-adiabatic multiphonon theory [12, 13] is totally inadequate for explaining the whole temperature dependence. Shown in Fig. 1 is an attempt to fit this theory to the fast phase of the present data. The parameters required are: energy gap, $\Delta E = 0.120$ eV; reorganization energy, $E_r = 4.64$ eV; $hw = 0.10$ eV (800 cm^{-1}); electron-exchange matrix element, $|HRP| = 205$ eV. The E_r is much too large according to present estimates of vibronic coupling and the value for $|HRP|$ is impossible. It was not possible to find any parameter values that would fit the slow phase. This agrees with Bixon and Jortner [14, 15] who rejected the single frequency version even on the basis of older less obvious data.

(2) In contradiction to refs. [14, 15], (and to [9]) the two parallel paths cannot, in general be assigned to two different

cytochromes. In *Rhodospseudomonas viridis* the two reactions (I) and (II) are well characterized, the different cytochromes being spectroscopically distinguishable. Yet they both give L-shaped Arrhenius plots which must be explained without any other cytochromes to assign.

(3) The mechanism for reaction (II) suggested in [3], i.e. equilibrium with C2 as an intermediate, cannot function at the lowest temperatures observed. The equilibrium partition of an electron between C2 and C3 would become too unfavorable. Further, even at room temperature, one would expect the free-energy difference between C2 and C3 (at least 0.31 eV) to add to any other activation energy, such as that for ET from C2 and C1, to give an apparent E, appreciably larger than the observed 0.36 eV.

(4) If an electron doesn't go from C3 to C1 by equilibration with C2 in reaction (II) it must tunnel the distance, which is about 20 Å, edge to edge. The presence of C2 in the path can greatly help the process. Without C2 the barrier to the electron tunneling has been estimated at values ranging from 2 to 6 eV. Suppose we take 3 eV. With C2 present the electron barrier height at the heme is about 0.3 eV (difference of the E.'s). It will be a little higher between the hemes but these two peaks will be quite narrow. The respective exponents in the Gamow factor for estimation of tunneling probabilities will then be something like -34.6 and -11.0 for a barrier width of 20 Å. The difference is 23.6 corresponding to a ratio of rates equal to 2×10^9 for C2 present vs. not present.

(5) Kihara and McCray [10] suggest an important role for water in ET to or from cytochromes. They examined many different cytochromes including soluble and nonphotosynthetic. In all cases removal of water prevented oxidation or reduction of the cytochromes. Replacement of H₂O restored original activity while substitution of D₂O for H₂O slowed the rates by a factor of about square root of 2. This was true at all temperatures tried, whether in a temperature dependent or a temperature independent range of temperature. Whatever causes the L-shaped temperature dependence (in Arrhenius plots) is affected equally at all temperatures by the presence of water and by the deuterium isotope effect. This seems to be peculiar to cytochromes as the primary reactions are little affected by drying.

(6) It is suggested in [14, 15] that the temperature independent paths result from lack of need for activation brought about by matching E_a to ΔE. This idea is good for the more primary reactions which must be fast and so the matching will be selected by evolution. There is suggestion that the non-heme iron in the reaction center plays the role of vibrator coupled to ET to Q_a [16]. However, the probability of such matching occurring by chance seems small when it can only be manifest under non-physiological conditions such as temperatures below 200K.

(7) It is also difficult to understand the suggestion [14, 15] that the vibronic coupling in the temperature independent

path ($E_r = 3500 \text{ cm}^{-1}$ or 0.43 eV) should be so much larger than in the temperature dependent path ($E_r = 900 \text{ cm}^{-1}$ or 0.12 eV). The tunneling electron has to pass the same vibrators in both paths.

(8) We need to find two paths, one dependent on the temperature and the other not, which are governed by some more general property of the system. Hales [17] and Parak et al. [18] suggested that protein conformational states generally become frozen in around 170K. The possibility that water in and around proteins undergoes some conformational change at such temperatures needs further investigation. Howard Robinson (personal communication) examining the X-ray structure of *Rhodopseudomonas viridis* [1] finds something like 50 water molecules in the crack between the cytochrome subunit and the BChl⁻ containing subunits and about 30 water molecules in the protein near the C1 to C3 hemes. In the membrane portion of the *Rhodopseudomonas viridis* structure where the fast primary ET's occur he finds at most about 5 water molecules. This accords with the expectation, based on the need to match E_r with E , that the vibronic coupling to ET should be kept small or well-regulated in this region [16].

(9) We suggest that the freezing in of the protein and water conformations needs further investigation. This suggestion has already been made in connection with cytochrome oxidation in *Chromatium vinosum* [19]. At higher temperatures all possible conformations are available through thermal agitation, but at various lower temperatures the possible conformations may become more restricted. The water in the protein would not be expected to like bulk water but may "freeze" at much lower temperature. Changes in vibrational modes available may occur. Particular dipole arrangements could be frozen in, which affect the barrier heights along the electron tunneling path so that changes in the electron exchange matrix element may also be considered.

ACKNOWLEDGEMENTS

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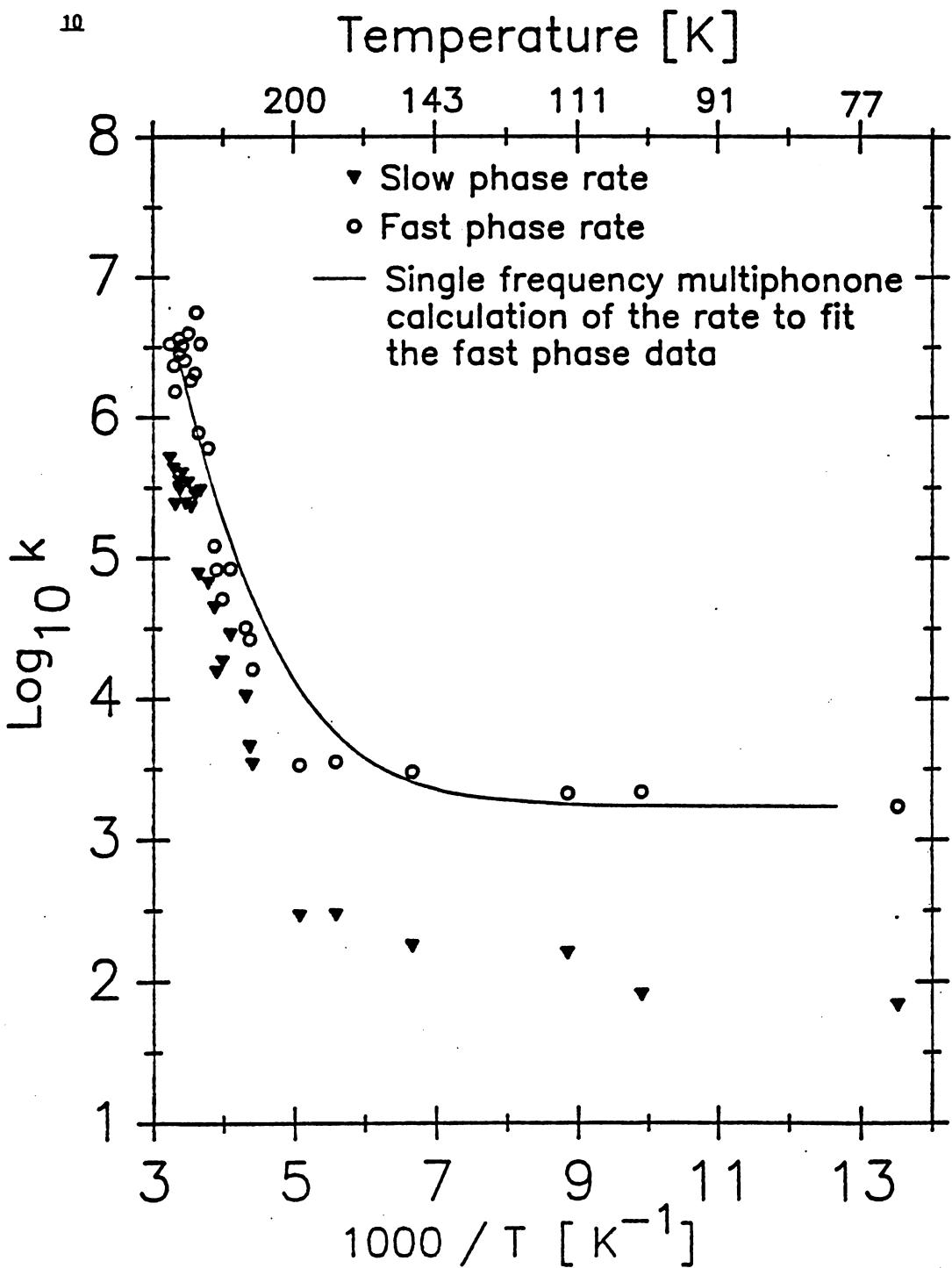
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TABLE 1. Temperature dependence of rates.

	Reaction (I) C1---e--->P [*] (=fast phase)	Reaction (II) C3---e--->C1 (=slow phase)	
ΔG	120	70	meV
Rate, k (295K)	3.3 ± 0.4	0.42 ± 0.07	10 ³ /sec
Half-time (295K)	0.18 ± 0.02	1.7 ± 0.3	μs
Rate, k (74K)	1.69 ± 0.20	0.069 ± 0.014	10 ³ /s
Half-time (74K)	0.41 ± 0.05	10 ± 2	ms
Transition temp.	220	210	K
E., high temp.	10.1	8.4	Kcal/mol
" " "	440	360	meV
E., low temp.	0.2	0.3	Kcal/mol
" " "	9	13	meV



3)

Preliminary sequence analysis for the "Foot and mouth disease virus" replicase protein has been done and the secondary structure of it predicted. Initial steps toward elucidating tertiary structure are taken.

Pages that follow outline the secondary structure predicted from the primary sequence. Rational is same as described in my trimestral report for the period November 15. to February 15. for the 2S storage protein.

Pages 12 to 16 have tabulated first part of the primary sequence of the protein (1 to 250) and secondary structure elements prediction according to Chou-Fasman algorithm. Page 17 is showing graphical presentation of the tabulated data. Cartoons given on page 18 and 19 are sequential secondary structure elements arrangement after initial and first iteration. Pages 20 and 21 show helical wheels, and helical cylinders with respect to amphipathy, respectively. Pages 22 to 30 give more insight on structure prediction analysis according to different algorithms used, as indicated on each plot (for detailed explanation see my latest report).

Pages 31 to 34 bring information about structure prediction for the second part of the protein sequence.

A quick analysis indicates plenty of amphipatic helical structures capable of forming hydrophobic nucleae with the possible environment accessible active site. The active site analysis is now in progress and will be accomplished for my final report.

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CHOU-FASMAN SECONDARY STRUCTURE PREDICTOR

NO.	AA	ACLASS	ALPHAV	BCLASS	BETAV	BNCL	TURN	ALPHA	BETA	TURN	HNUCL	PRCTN	PHASE
-----	----	--------	--------	--------	-------	------	------	-------	------	------	-------	-------	-------

1	G	B	0.98	b	1.24		0.81		?		N	E	L
2	L	H	0.99	h	1.25	N	0.81		?		N	E	W
3	I	h	0.99	H	1.24		0.82		?		N	E	W
4	V	h	0.99	H	1.19	N	0.89		?		N	E	W
5	D	I	1.01	B	1.12	N	0.97		?				
6	T	i	1.05	h	1.07		1.03			!**		E	W
7	R	i	1.08	i	0.98		1.00		!	**		H	W
8	D	I	1.11	B	0.89		0.93		!		N	H	W
9	V	h	1.15	H	0.90		0.84		!		N	H	W
10	E	H	1.18	B	0.87		0.79		!		N	H	W
11	E	H	1.18	B	0.91		0.76		!		N	H	W
12	R	i	1.17	i	1.00		0.74		!		N	H	W
13	V	h	1.15	H	1.10		0.72		!		N	H	W
14	H	I	1.13	i	1.10	N	0.71		?		N	H	W
15	V	h	1.11	H	1.16	N	0.74		?		N	E	W
16	M	H	1.10	h	1.12		0.80		!		N	H	W
17	R	i	1.11	i	1.07	N	0.86		?		N	H	W
18	K	h	1.10	b	1.01		0.90		!		N	H	W
19	T	i	1.09	h	0.98		0.90		!		N	H	W
20	K	h	1.07	b	0.94		0.89		!		N	H	W
21	L	H	1.06	h	0.98		0.90		!		N	H	C
22	A	H	1.05	i	0.99		0.91		!	**	N	H	W
23	P	B	1.02	B	1.00	N	0.90		?		N	N	L
24	T	i	1.01	h	1.03		0.88		!		N	N	L
25	V	h	1.01	H	1.08		0.87		?		N	N	L
26	A	H	0.99	i	1.08		0.88		!	*	N	N	L
27	H	I	0.95	i	1.10		0.91		?				
28	G	B	0.92	b	1.10		0.96		?				
29	V	h	0.89	H	1.10		1.03		?				
30	F	h	0.84	h	1.06		1.12		?	*			
31	N	b	0.78	i	1.02		1.22		?	!**			
32	P	B	0.77	B	0.95		1.28		?	*****			
33	G	B	0.81	b	0.91		1.28		!	*			
34	F	h	0.85	h	0.87		1.24		!	*			
35	G	B	0.90	b	0.85		1.14		!				
36	P	B	0.96	B	0.84		1.04	!	!	**			
37	A	H	1.02	i	0.87		0.95				N		
38	A	H	1.04	i	0.88		0.95						
39	L	H	1.01	h	0.87		1.04	!					
40	S	i	0.98	b	0.84		1.17	!		**			
41	N	b	0.97	i	0.82		1.27			**			
42	K	h	0.93	b	0.80		1.29			***			
43	D	I	0.92	B	0.78		1.25						
44	P	B	0.93	B	0.78		1.17			****			
45	R	i	0.95	i	0.81		1.11			!			
46	L	H	0.97	h	0.86		1.07						
47	N	b	0.99	i	0.92		1.05			**			
48	E	H	1.00	B	0.98		0.99			**			
49	G	B	1.03	b	1.06		0.90		?				
50	V	h	1.06	H	1.11		0.83	?	!		N		
51	V	h	1.09	H	1.14		0.80	?	!		N		
52	L	H	1.12	h	1.14		0.80	!	!		N		

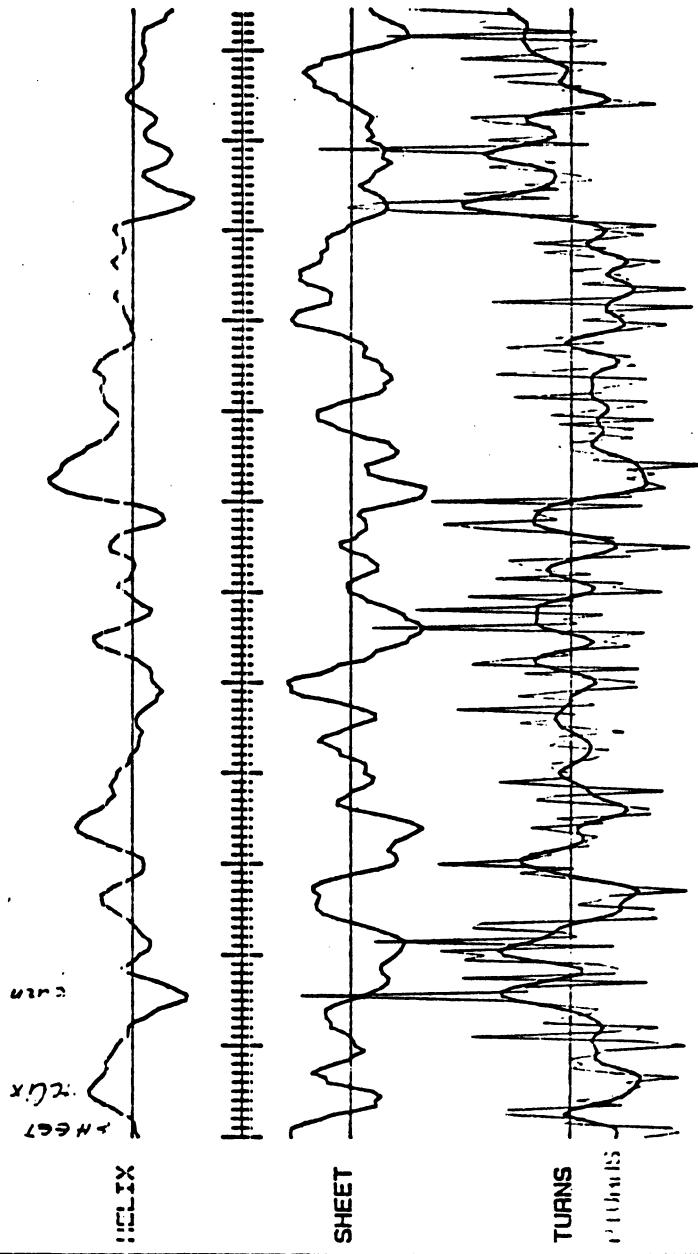
53 D	I	1.13	B	1.13	N	0.78	!	!	!	N	E
54 E	H	1.13	B	1.12		0.75	!	!	!	W	W
55 V	h	1.10	H	1.15		0.72	?	?	?	W	W
56 I	h	1.07	H	1.15		0.77	?	?	?	W	W
57 F	h	1.03	H	1.12	N	0.86				W	W
58 S	h	0.99	b	1.06		0.97				W	W
59 R	i	0.96	i	0.98		1.06				W	W
60 H	I	0.95	i	0.89		1.14				W	W
61 K	h	0.95	b	0.82		1.20				W	W
62 G	B	0.96	b	0.81		1.21				W	W
63 D	B	1.00	B	0.82		1.17	!			W	W
64 T	I	1.05		0.85		1.08				W	W
65 K	i	1.10	b	0.83		1.00	!			W	W
66 M	h	1.14	b	0.82		0.95	!			W	W
67 S	H	1.19	b	0.77		0.95	!			W	W
68 A	H	1.23	i	0.74		0.97	!			W	W
69 E	H	1.24	B	0.70		0.97	!			W	W
70 D	I	1.22	B	0.75		0.94	!			W	W
71 K	h	1.21	b	0.81		0.87	!			W	W
72 A	H	1.18	i	0.90		0.79	!			W	W
73 L	H	1.14	h	0.99		0.77	!			W	W
74 F	H	1.11	h	1.05	N	0.80	?			W	W
75 R	h	1.10	i	1.05		0.86	?			W	W
76 R	i	1.07	i	1.02		0.91	!			W	W
77 C	i	1.08	h	1.00		0.93	!			W	W
78 A	H	1.09	i	0.95		0.95	!			W	W
79 A	H	1.08	i	0.91		0.99	!			W	W
80 D	I	1.07	B	0.90		1.03	!			W	W
81 Y	B	1.06	H	0.94		1.05	!			W	W
82 A	H	1.04	i	0.94		1.03	!			W	W
83 S	i	1.01	b	0.94		0.99		?		W	W
84 R	i	1.01	i	0.99		0.96				W	W
85 L	H	1.00	h	1.04		0.93				W	W
86 H	I	0.98	i	1.04		0.92	?			W	W
87 S	H	0.97	b	1.09	N	0.92				W	W
88 V	H	0.97	H	1.13		0.93				W	W
89 L	H	0.97	h	1.11		0.96				W	W
90 G	B	0.96	b	1.05	N	1.00				W	W
91 T	B	0.98	h	1.01		1.04				W	W
92 A	I	0.98	i	0.96		1.05				W	W
93 N	H	0.98	i	0.90		1.07				W	W
94 A	B	0.97	i	0.90		1.06				W	W
95 P	B	0.95	B	0.95		1.04				W	W
96 L	B	0.93	h	1.01		1.02				W	W
97 S	H	0.90	b	1.09		1.00				W	W
98 I	H	0.90	H	1.20		1.00				W	W
99 Y	H	0.88	H	1.25		0.96				W	W
100 G	B	0.90	b	1.24	N	0.93				W	W
101 V	H	0.92	H	1.27		0.90				W	W
102 I	H	0.92	H	1.23		0.92				W	W
103 K	h	0.92	b	1.12		0.98				W	W
104 G	B	0.93	b	1.08		1.07				W	W
105 V	B	0.96	H	1.05		1.14				W	W
106 D	B	0.98	B	0.95		1.15				W	W
107 G	B	1.04	b	0.91		1.12	!			W	W
108 L	H	1.09	h	0.90		1.03	!			W	W
109 D	I	1.14	B	0.83		0.94	!			W	W
110 A	H	1.16	i	0.77		0.91	!			W	W
111 M	H	1.16	h	0.76		0.95	!			W	W
112 E	H	1.12	B	0.72		1.04	!			W	W

113	P	B	1.05	B	0.70	1.11	!	*****	N	T	W
114	D	I	1.02	B	0.72	1.15	!	!	!	T	W
115	T	i	0.97	h	0.77	1.14	!	!	!	T	W
116	A	H	0.93	i	0.78	1.14	!	!	!	T	W
117	P	B	0.92	B	0.83	1.14	!	!***	!	T	W
118	S	i	0.96	b	0.87	1.14	!	!	!	T	W
119	L	H	0.98	h	0.93	1.08	!	!	!	T	W
120	P	B	1.00	B	0.95	1.00	!	**	N	N	W
121	W	h	1.03	h	1.01	N	0.93	!	N	N	W
122	A	H	1.06	i	1.01	N	0.90	!	N	N	W
123	L	H	1.05	h	1.02	N	0.95	!	N	N	W
124	Q	h	1.01	h	0.98	1.02	!	**	N	N	W
125	G	B	1.00	b	0.94	1.08	!	!	N	N	W
126	K	h	0.99	b	0.89	1.10	!	!	N	N	W
127	R	i	0.99	i	0.89	1.08	!	!**	N	N	W
128	R	i	1.00	i	0.92	1.02	!	!	N	N	W
129	G	B	1.03	b	0.94	0.94	!	!	N	N	W
130	A	H	1.09	i	1.00	0.86	?	!	N	N	W
131	L	H	1.10	h	1.04	0.82	!	!	N	N	W
132	I	h	1.09	H	1.04	N	0.83	!	N	N	W
133	D	I	1.07	B	0.98	0.90	!	!	N	N	W
134	F	h	1.05	h	0.97	1.01	!	!**	N	N	W
135	E	H	0.98	B	0.94	1.10	!	!***	N	N	W
136	N	b	0.90	i	0.94	1.15	!	*	N	N	W
137	G	B	0.87	b	0.95	1.15	!	*	N	N	W
138	T	i	0.88	h	0.97	1.14	?	*	N	N	W
139	V	h	0.91	H	0.96	1.12	!	*	N	N	W
140	G	B	0.97	b	0.87	1.09	!	!	N	N	W
141	P	B	1.09	B	0.78	1.02	!	!***	N	N	W
142	E	H	1.20	B	0.71	0.91	!	!	N	N	W
143	A	H	1.27	i	0.70	0.79	!	!	N	N	W
144	E	H	1.31	B	0.69	0.71	!	!	N	N	W
145	A	H	1.34	i	0.78	0.69	!	!	N	N	W
146	A	H	1.35	i	0.87	0.70	!	!	N	N	W
147	L	H	1.32	h	0.93	N	0.71	!	N	N	W
148	K	h	1.30	b	0.93	0.72	!	!	N	N	W
149	L	H	1.29	h	0.94	0.73	!	!	N	N	W
150	M	H	1.27	h	0.88	0.76	!	!	N	N	W
151	E	H	1.24	B	0.83	0.82	!	*	N	N	W
152	K	h	1.21	b	0.81	0.87	!	*	N	N	W
153	R	i	1.19	i	0.83	0.91	!	*	N	N	W
154	E	H	1.15	B	0.85	0.92	!	*	N	N	W
155	Y	b	1.11	H	0.94	0.90	!	*	N	N	W
156	K	h	1.09	b	0.99	0.87	!	*	N	N	W
157	F	h	1.08	h	1.05	N	0.87	!	N	N	W
158	A	H	1.06	i	1.08	N	0.89	!	N	N	W
159	C	i	1.05	h	1.14	N	0.90	?	N	N	W
160	Q	h	1.06	h	1.14	N	0.88	?	N	N	W
161	T	i	1.07	h	1.14	N	0.85	?	N	N	W
162	F	h	1.10	h	1.08	N	0.85	?	N	N	W
163	L	H	1.12	h	1.04	0.88	!	*	N	N	W
164	K	h	1.12	b	0.96	0.91	!	*	N	N	W
165	D	i	1.13	B	0.89	0.92	!	*	N	N	W
166	E	H	1.14	B	0.86	0.91	!	*	N	N	W
167	I	h	1.12	H	0.87	0.91	!	*	N	N	W
168	R	i	1.12	i	0.83	0.92	!	*	N	N	W
169	P	B	1.13	B	0.84	0.91	!	**	N	N	W
170	M	H	1.16	h	0.87	0.88	!	!	N	N	W
171	E	H	1.15	B	0.88	0.82	!	!	N	N	W
172	K	h	1.14	b	0.89	0.81	!	!	N	N	W

173	V	h	1.11	H	0.95	0.84	!	N	H
174	R	i	1.09	i	0.95	0.92	!	N	H
175	A	H	1.03	i	0.93	1.00	!	N	H
176	G	B	1.00	b	0.96	1.03	!	N	T
177	K	B	0.99	b	1.01	0.99	!	N	E
178	T	h	0.99	h	1.05	0.90	?	N	E
179	R	i	1.01	i	1.13	N	?	N	E
180	I	h	1.01	H	1.23	N	?	N	E
181	V	h	1.02	H	1.25	N	?	N	E
182	D	I	1.03	B	1.23	N	?	N	E
183	V	H	1.04	H	1.22	0.86	?	N	E
184	L	H	1.04	h	1.15	0.86	?	N	E
185	P	B	1.06	B	1.08	0.84	?	N	E
186	V	B	1.07	H	1.09	0.81	!	N	E
187	E	H	1.07	B	1.08	0.76	!	N	E
188	H	H	1.05	i	1.11	N	!	N	E
189	I	H	1.04	H	1.18	N	?	N	E
190	L	H	1.05	h	1.21	N	?	N	E
191	Y	b	1.05	H	1.21	N	?	N	E
192	T	i	1.05	h	1.21	N	?	N	E
193	R	H	1.07	i	1.17	N	?	N	E
194	M	H	1.09	h	1.13	N	?	N	E
195	M	H	1.06	h	1.12	N	!	N	E
196	I	H	1.04	H	1.13	N	!	N	E
197	G	B	1.03	b	1.10	N	?	N	E
198	R	i	1.04	i	1.10	N	?	N	E
199	F	h	1.04	h	1.10	0.91	!	N	E
200	C	i	1.06	h	1.08	N	!	N	E
201	A	H	1.06	i	1.03	N	!	N	E
202	Q	H	1.07	h	1.01	N	!	N	E
203	M	H	1.02	h	0.97	1.01	!	N	E
204	H	H	0.93	i	0.92	1.16	!	N	E
205	S	I	0.86	b	0.87	1.33	***	N	E
206	N	i	0.80	i	0.85	1.44	****	N	E
207	N	b	0.77	b	0.86	1.45	*****	N	E
208	G	B	0.75	b	0.87	1.36	!	N	E
209	P	B	0.79	B	0.90	1.24	**	N	E
210	Q	B	0.86	h	0.94	1.15	*	N	E
211	I	h	0.90	H	0.97	1.10	*	N	E
212	G	B	0.93	b	0.93	1.07	!	N	E
213	S	i	0.96	b	0.91	1.06	*	N	E
214	A	H	0.95	i	0.90	1.08	**	N	E
215	A	H	0.90	i	0.87	1.14	**	N	E
216	G	B	0.86	b	0.84	1.24	*	N	E
217	C	i	0.85	h	0.87	1.33	*	N	E
218	N	b	0.84	i	0.87	1.36	**	N	E
219	P	B	0.85	B	0.86	1.30	****	N	E
220	D	I	0.89	B	0.88	1.20	!	N	E
221	V	H	0.94	H	0.93	1.12	**	N	E
222	D	I	0.95	B	0.91	1.07	*	N	E
223	R	i	0.95	i	0.92	1.08	*	N	E
224	Q	h	0.93	h	0.94	1.12	!	N	E
225	R	i	0.91	i	0.92	1.18	**	N	E
226	S	b	0.90	b	0.91	1.19	**	N	E
227	G	B	0.93	b	0.94	1.13	!	N	E
228	T	i	0.96	h	0.97	1.01	*	N	E
229	H	I	1.00	i	1.01	N	?	N	E
230	L	H	1.02	h	1.05	0.84	?	N	E
231	A	H	1.03	i	1.07	N	?	N	E
232	Q	H	1.01	h	1.11	N	?	N	E

233	Y	b	0.98	H	1.15	1.03			E
234	R	b	0.96	i	1.15	N	1.05	?	EE
235	N	b	0.96	i	1.17	N	1.03	?	EE
236	V	h	0.96	H	1.20		1.02	?	EE
237	W	h	0.96		1.18	N	1.01	?	EE
238	D	I	0.97	B	1.12		1.06	?	E
239	V	h	0.96	H	1.12		1.10	?	E
240	D	I	0.95	B	1.04		1.14	?	T
241	Y	b	0.95	H	0.97		1.17	!	T
242	S	i	0.96	b	0.88		1.18	!	T
243	A	H	0.96	i	0.83		1.20	!	T
244	P	B	0.96	B	0.76		1.19	****	C
245	D	I	0.96	B	0.77		1.19	!	T
246	A	H	0.95	i	0.79		1.18	**	C
247	N	b	0.91	i	0.83		1.18	**	T
248	H	I	0.87	i	0.87		1.21	**	T
249	C	i	0.86	h	0.91		1.23	!	T
250	S	i	0.84	b	0.92		1.26	****	T

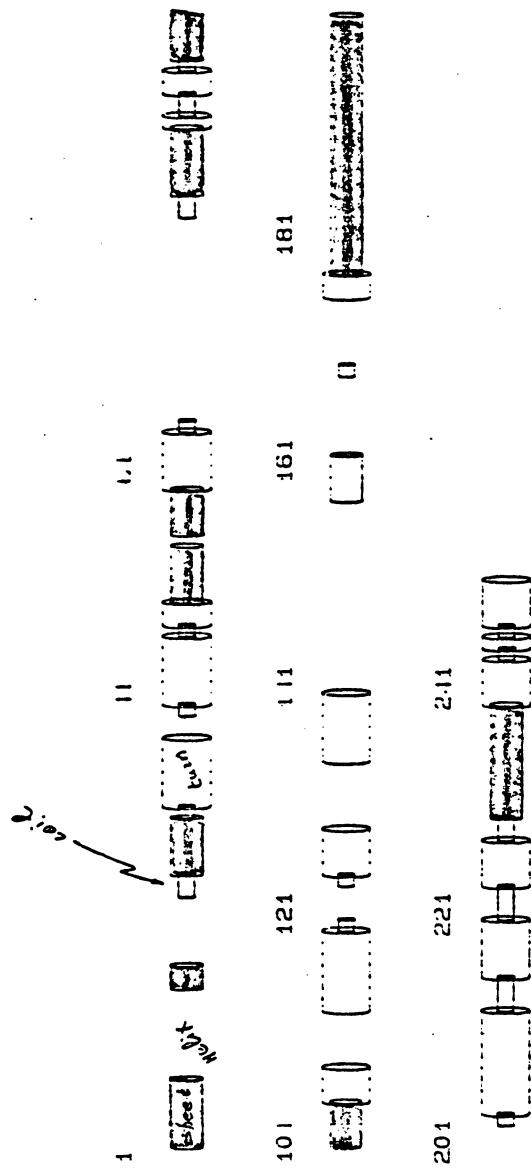
CHOU-FASMAN SECONDARY STRUCTURE PREDICTOR FOR c:\info\tanuri.dat



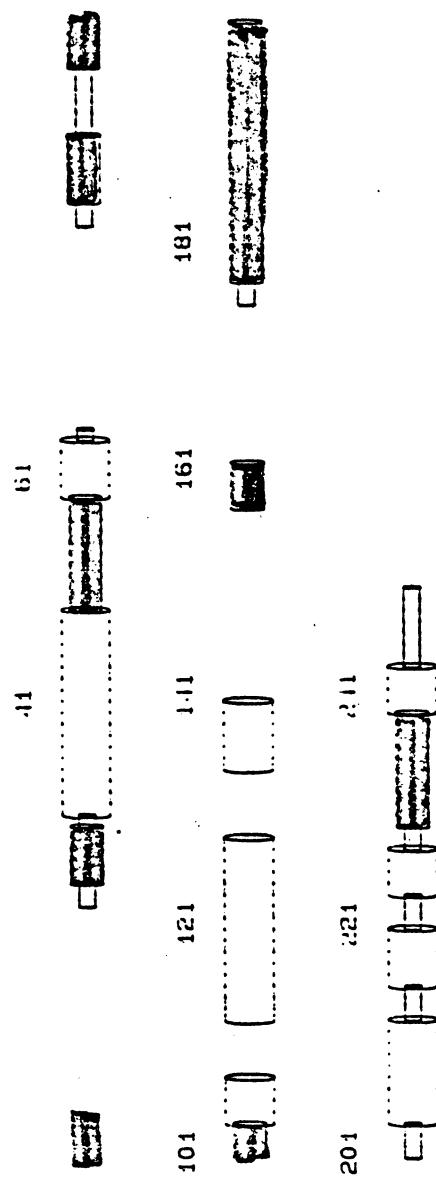
Lamur:

Based on Consensus = Eisenberg prediction Algorithm

18



1st iteration term (n)



blue -	b <u>a</u> sic (+)
red -	a <u>c</u> idic (-)
yellow -	i <u>n</u> verna <u>l</u>
green -	am <u>b</u> ival <u>e</u> al (neutral)

ADVEERVHIVMARKTKLA
7 - 22

82 -

KMSAEUKALFARCAADYA

The diagram consists of two separate clusters of twelve numbered circles each, arranged in a roughly circular pattern. The top cluster is labeled 'LNAME' vertically to its left. Dashed lines connect circle 1 to circle 2, circle 5 to circle 6, and circle 10 to circle 11. The bottom cluster has dashed lines connecting circle 1 to circle 2, circle 5 to circle 6, and circle 10 to circle 11. The circles are numbered 1 through 12.

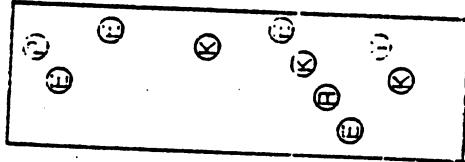
129 - 134
GAI TNE

PEAEAAALKLMEKREYKF

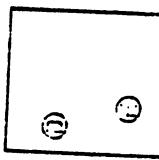
162 - 175
FLKDEIAPWEVKKA

四

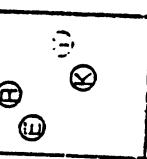
141



108

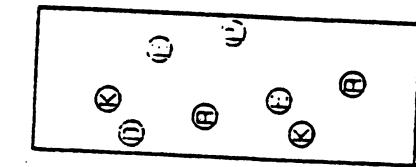


112

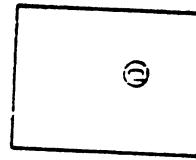


157

162

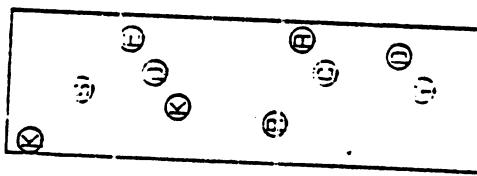


129



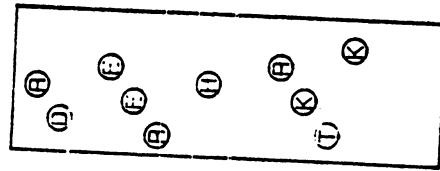
134

65



82

7



22

Turns

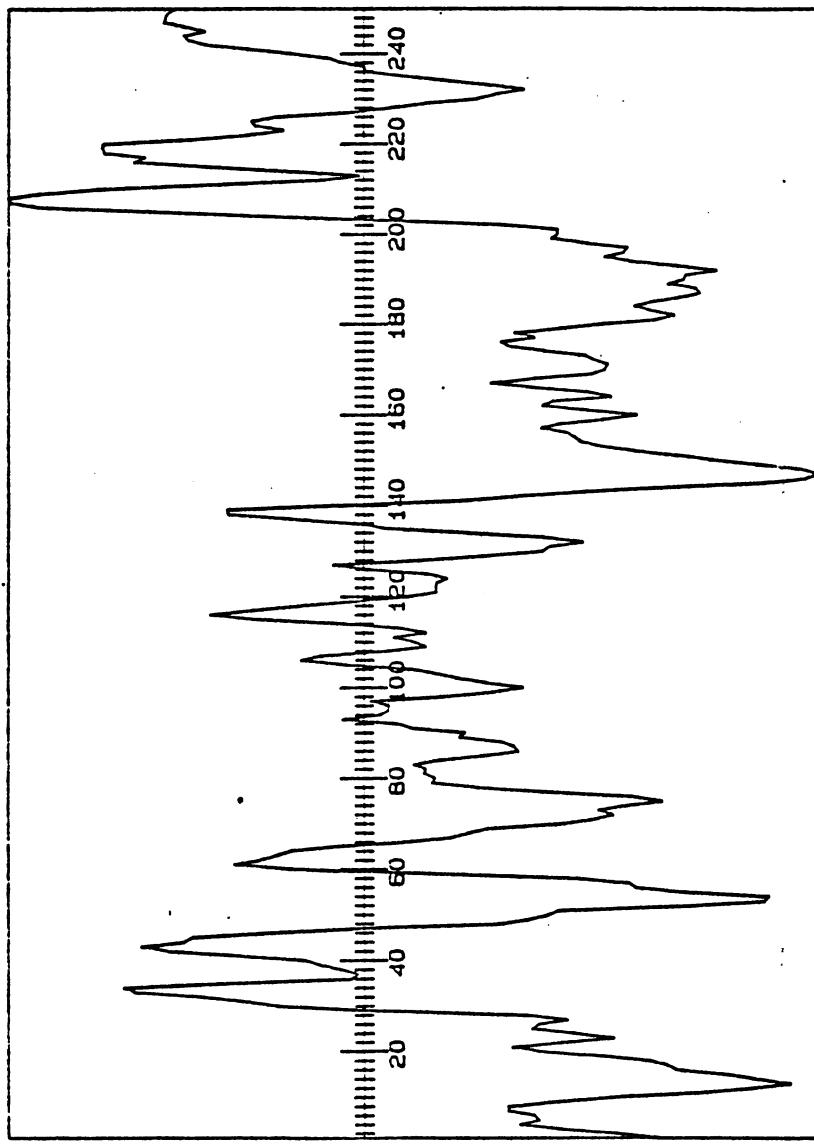
22

1.326

1.049

0.695

Chou-Fasman turns, span 1.3, smoothed 7×1



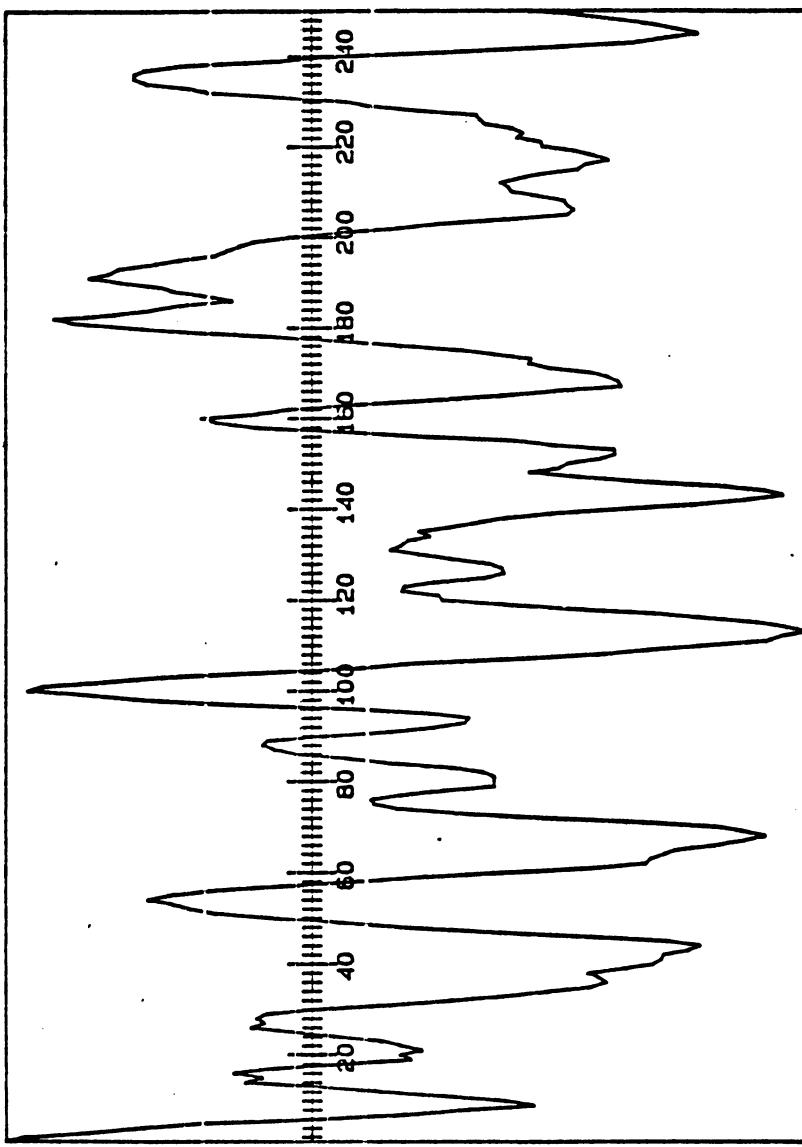
Chou-Fasman betasheet. span is 5. smoothed 7 x 1

tanur1

1.249

1.049

0.727

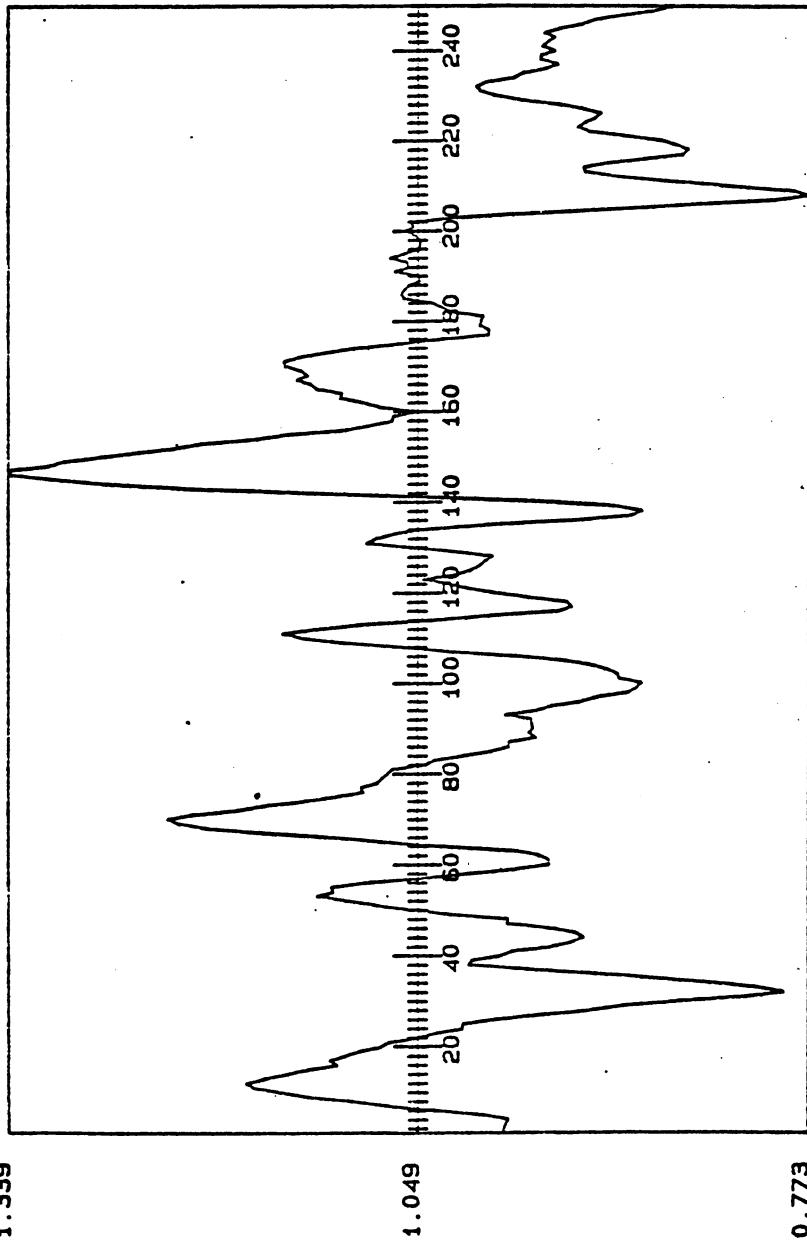


tanur1

1.339

1.049

0.773



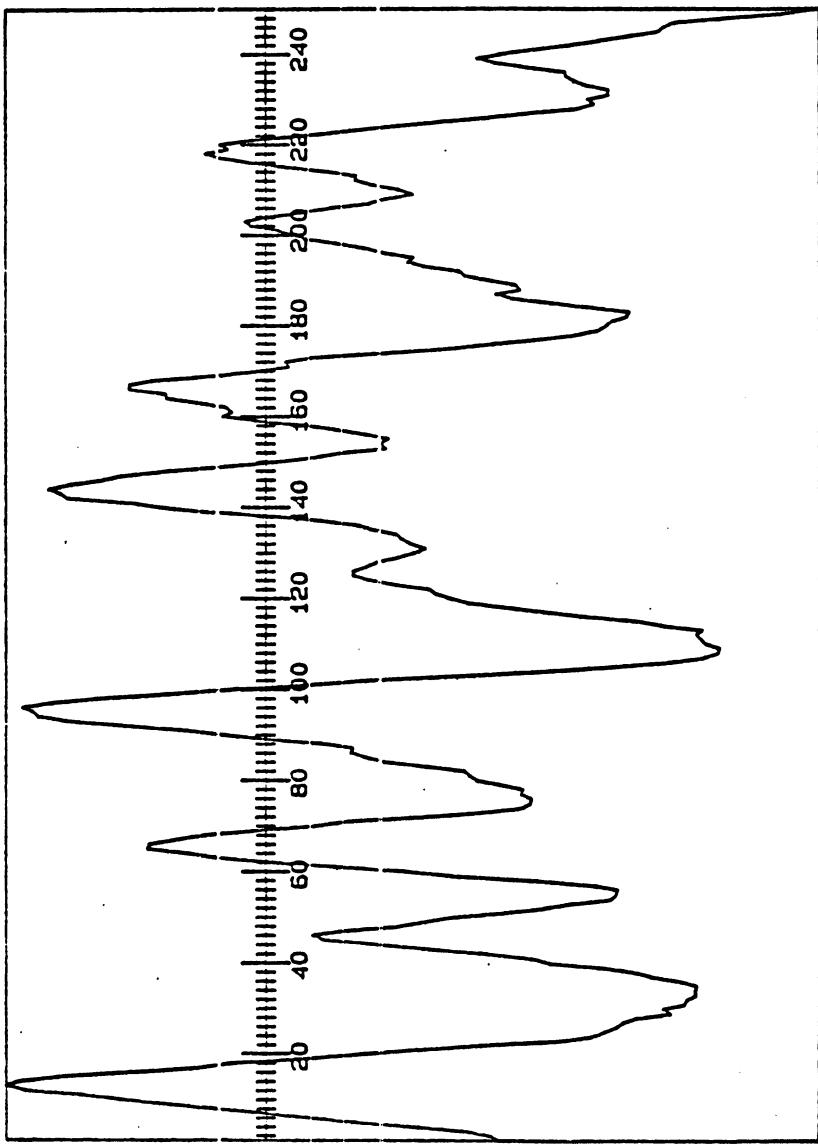
Chou-Fasman α -helix, span is 5, smoothed 7×1

tanur 1

1.378

1

0.201



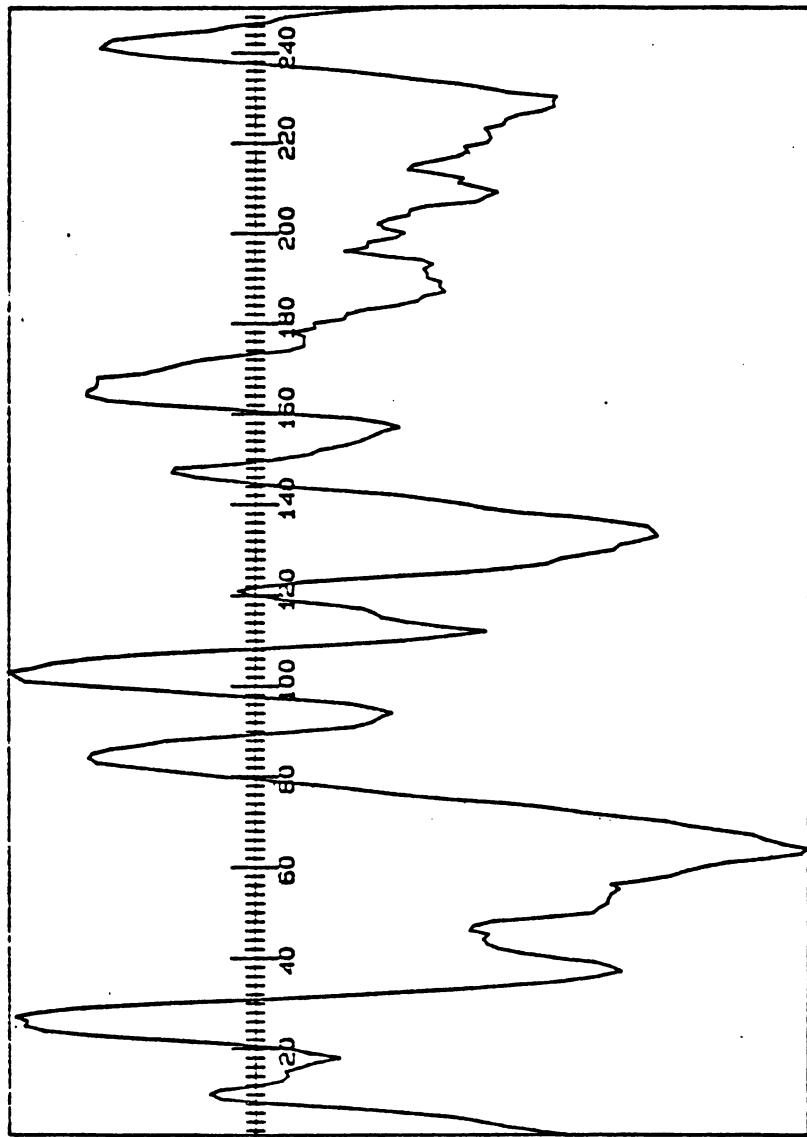
Amphiphilicity at 170 degrees. span is 11. smoothed 11 x 1

tenth

1.332

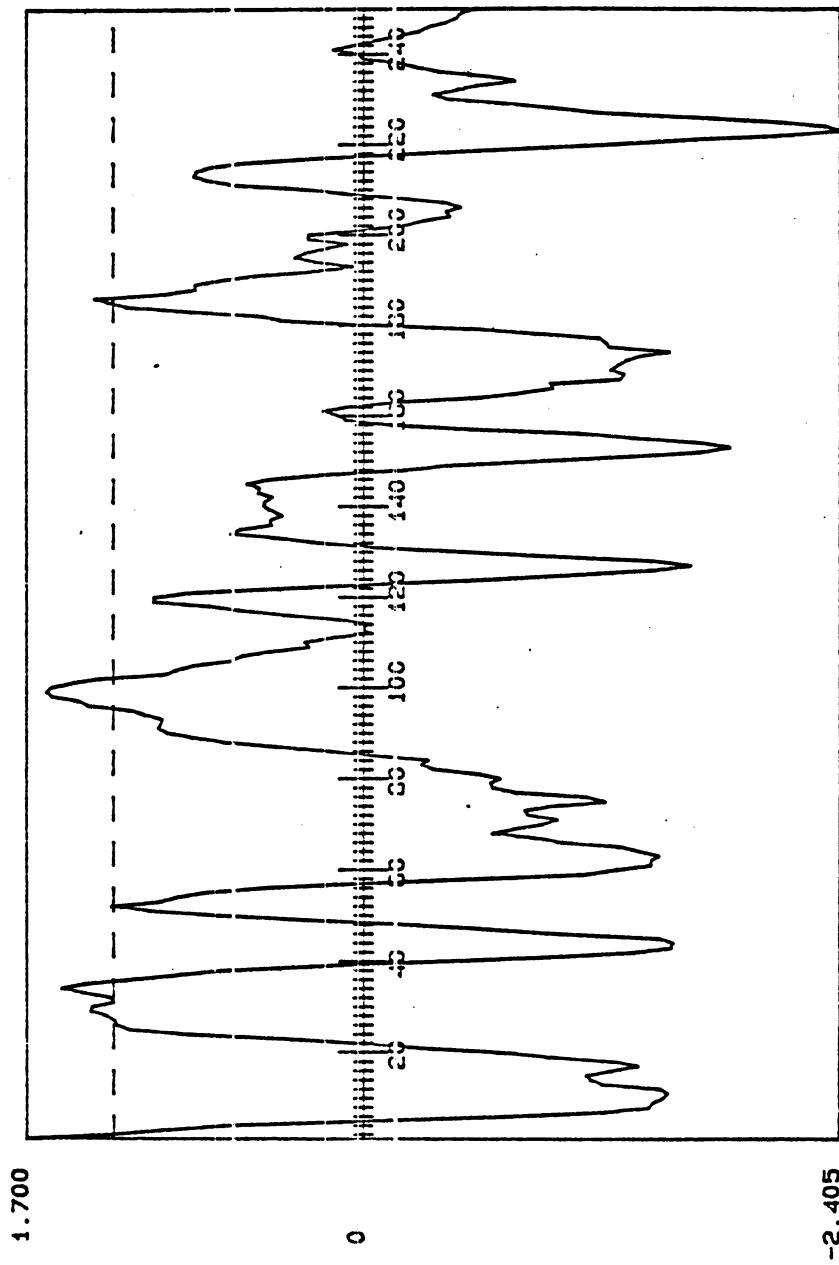
1

0.254



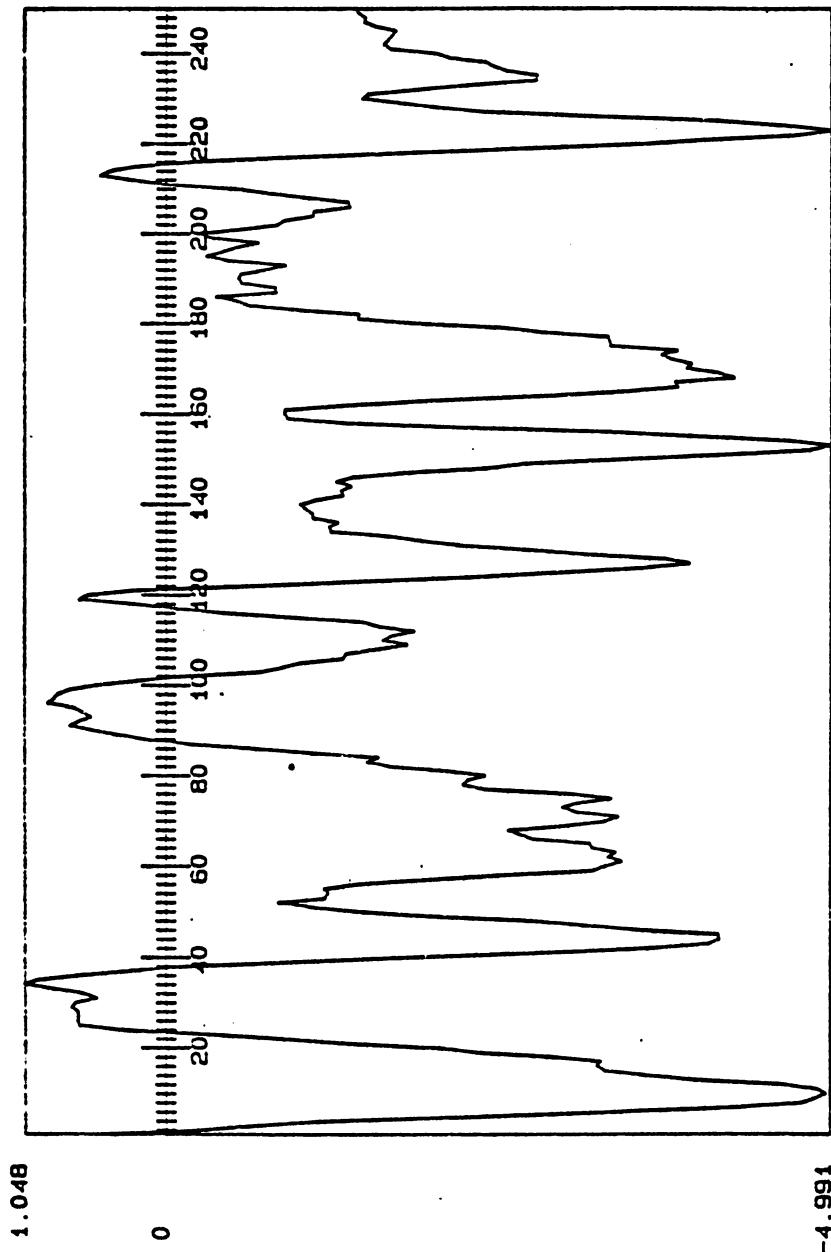
Amplitude at 100 degrees, span is 11, smoothed 11×1
(kyre)

tanuri



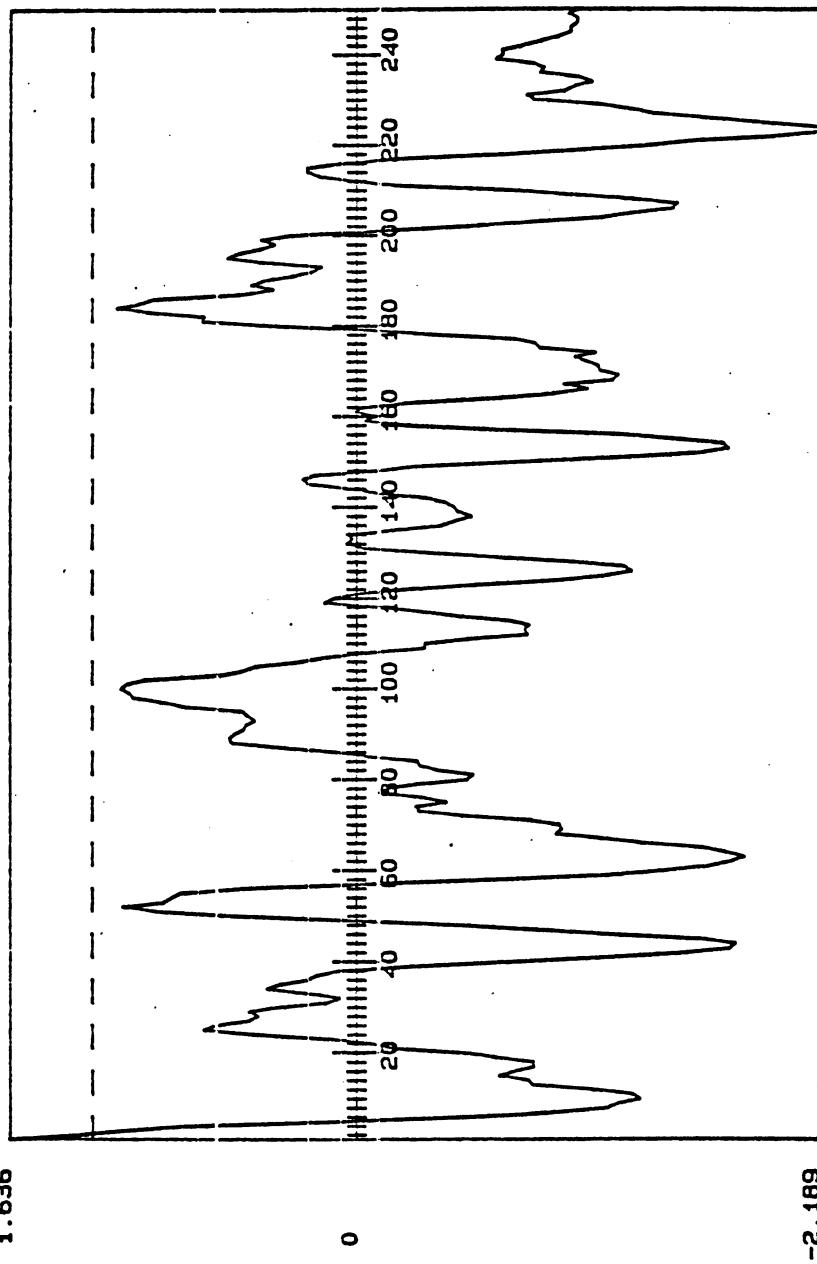
Eisenberg consensus hydropathy. span is 7, smoothed 7 x 1

tanur1



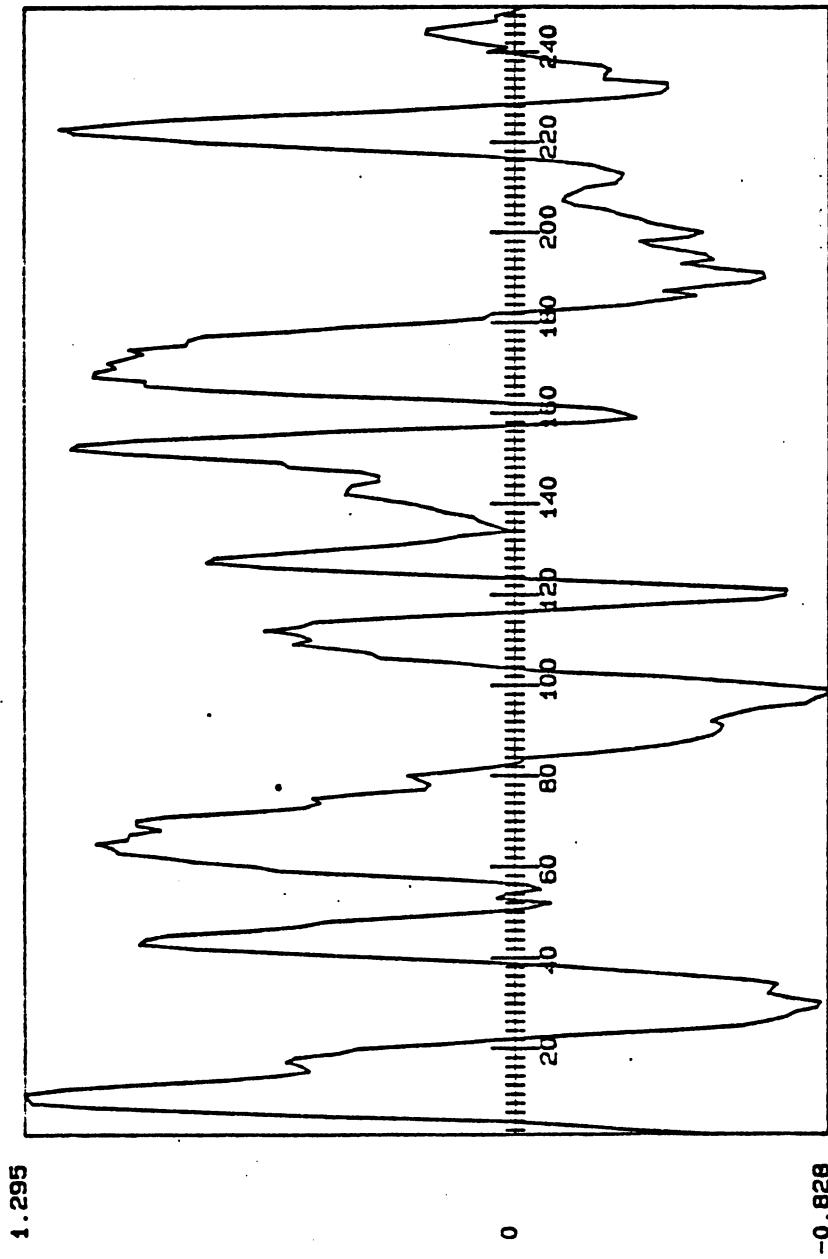
GES hydropathy. span is 7. smoothed 7 x 1

Kyte hydroopathy. span is 7. smoothed 7.x 1



tanur!

tanuri

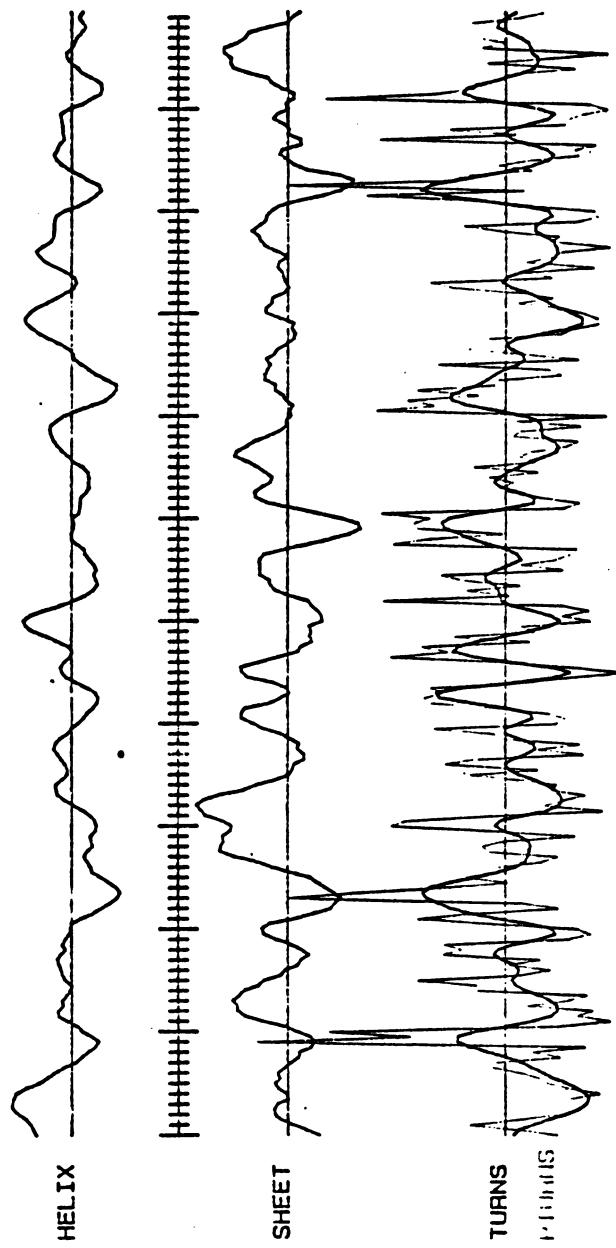


Hopp hydrophilicity. span is 7. smoothed 7 x 1

70022 24-

1	D	A	M	N	I	M	F	E	E	V	F	R	T	E	F	G	F	H	P	N	
21	A	G	W	I	L	K	T	L	V	N	T	E	Y	A	Y	E	N	K	R	I	
41	T	V	E	G	G	M	P	S	G	C	S	A	T	S	I	V	N	T	I	L	
61	N	N	I	Y	V	I	Y	A	I	R	R	H	Y	E	G	V	E	L	D	T	
81	Y	T	M	I	S	Y	G	D	D	I	V	V	A	S	D	Y	D	L	D	F	
101	E	A	L	K	P	A	F	F	Y	S	L	G	Q	T	I	T	P	A	D	K	S
121	D	K	G	F	V	L	G	H	S	I	T	D	V	T	F	L	K	R	H	F	
141	H	M	D	Y	G	T	G	F	Y	K	P	V	M	T	S	K	T	L	E	A	
161	I	L	S	F	A	R	R	G	T	I	Q	E	K	L	I	S	V	A	G	L	
181	A	V	H	S	G	P	D	E	Y	R	R	F	F	E	P	F	Q	G	L	F	
201	E	I	P	S	Y	R	S	L	Y	L	R	W	V	N	A	V	C	G	R	A	

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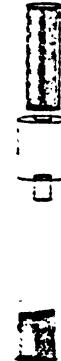
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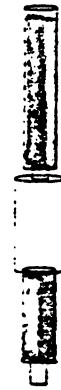
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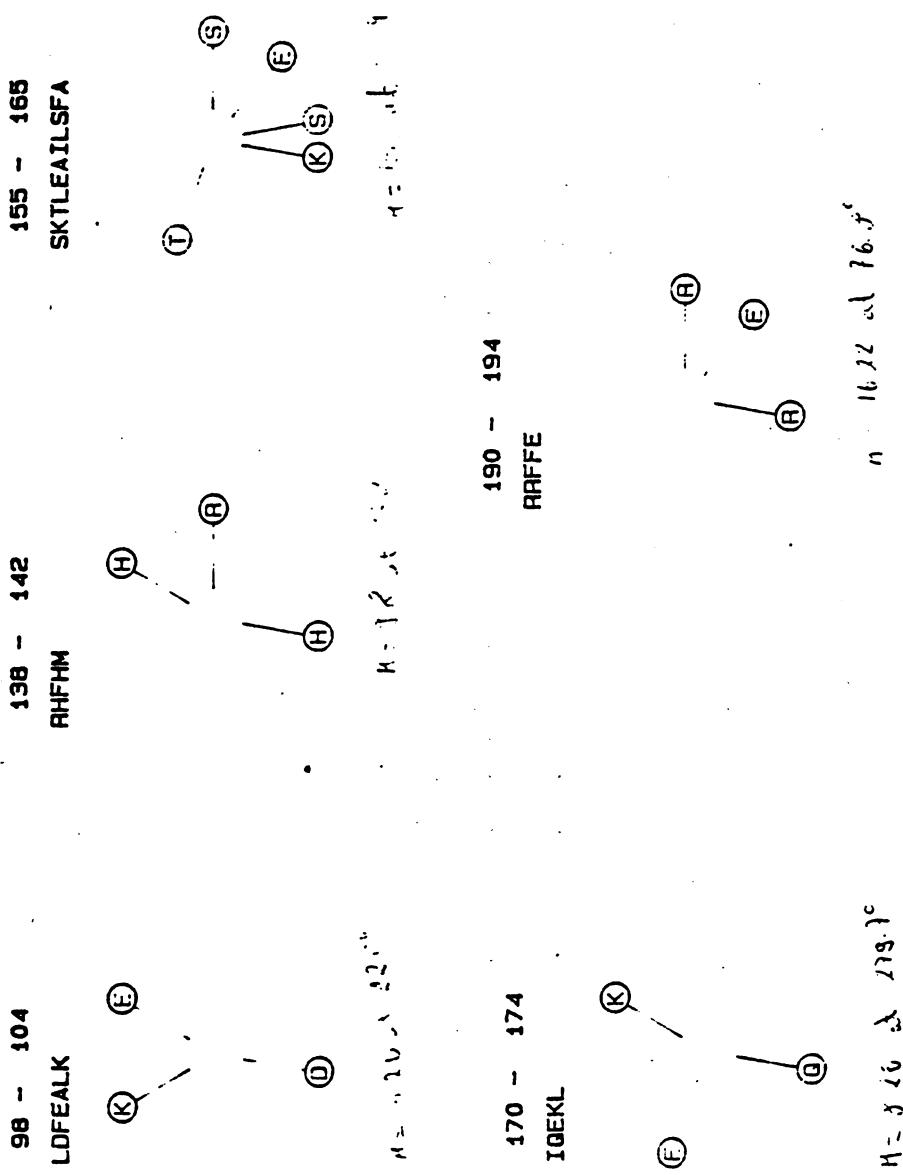
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4) Report on visit to the University of Illinois

All of the major objectives for the trip to USA as outlined in my "Proposal and justification for the short visit to "University of Illinois", "Silicon Graphics" and "SLM Aminco", submitted earlier to CENARGEN scientific board, have been successfully accomplished. I will refer to that document because of the limited space here.

Subject I)-1). Complete computer system has been agreed to be purchased through SLM Aminco as the controller for their DW-2000 spectrophotometer, under very favorable conditions. "PRO FORMA INVOICE" from SLM Aminco is available in my files.

Subject I)-2). ASYST software package is also agreed to be purchased through the SLM Aminco by the same order.

Subject I)-3). MMS molecular presentation package has been purchased from Univ. of California, San Diego, through my connection with Univ. of Illinois and generous help of Dr. Howard Robinson who facilitated purchase under USA university prices. Namely we have paid this package \$300.00, while regular price is \$10,000.00. Similarly, we got MIDAS presentation package for \$200.00, while regular price amounts to \$15,000.00. Complete documentation and manuals are in my files.

Subject II). Visit to "Silicon graphics" was very fruitful: I have got numerous advises from their top engineers

for the initial setup procedure, as well as couple of procedures not regularly packed with the initial purchase. I was able to get "Silicon graphics" "customer serial number approval" on purchase order through "University of Illinois" for the molecular graphics presentation software packages "MMS" and "MIDAS" (to be used on the workstation itself).

Subject III). "SLM Aminco" dual wavelength DW-2000 spectrophotometer, with extended sensitivity range up to 1050 nm and French pressure cell are solely produced by "SLM AMINCO". The manufacturer is located in vicinity of University of Illinois. We obtained complete system specification and price list according to description we have submitted to them. Thanks to generous help of Dr. Matheis with whom I had earlier contact, some additional "non-SLM" components were included in the final offer to meet our needs. Again, complete documentation is available in my files.

Subjects IV) and V) Pro forma invoice was obtained for purchase through IIICA, and therefore under more favorable conditions.

Subject VI). Portable Joliot-type spectrophotometer was tentatively ordered from "Kinetic instruments" in Urbana, Illinois. This apparatus is now built and ready for check-up in the laboratory of Prof. Antony Crofts. The detailed description and purchase justification for this versatile instrument is given in the project proposal. To back-up the necessity of getting this instrument now, I would like to state just two areas that CENARGEN could be very interested in pursuing research in Brasil:

Drought stress in crops and heavy metal pollution induced stress in plants. Also, we may use it for different mutant specification of photosynthetic tissue cultures or bacterial cultures. Invoice was obtained.

Subject VII) Necessary consultations with Dr. Don DeVault and Dr. Colin Wraight about project development were done, as well as final revision on the scientific paper (shown on pages 3 to 11 of this report). This is the first scientific paper from the protein engineering laboratory at CENARGEN.

In addition, I was able to obtain from Dr. Howard Robinson complete x-ray structure data bank, known as Brookhaven protein data-bank, with about 500 files containing all up-to-date available protein structures obtained by x-ray spectroscopy.

Also I brought invoice for the digital oscilloscope from LeCroy, necessary for the complete biophysics laboratory (as described in my initial project proposal) and some biological material to replace the old one, brought also by me in November last year.

We should use all of the obtained offers as soon as possible, due to the fact that they have limited time validity, and are still favorable options (since bargaining was done assuming my position in Univ. of Illinois)

Financial report is done separately and submitted to IICA director Dr. Horacio Stagnho (with the copy to Dr. Maria Jose Sampaio).

Programa II. Geração e Transferência de Tecnologia

O Programa de Geração e Transferência de Tecnologia é a resposta do IICA a dois aspectos fundamentais: (i) o reconhecimento, por parte dos países e da comunidade técnico-financeira internacional, da importância da tecnologia para o desenvolvimento produtivo do setor agropecuário; (ii) a convicção generalizada de que, para aproveitar plenamente o potencial da ciência e da tecnologia, é necessário que existam infra-estruturas institucionais capazes de desenvolver as respostas tecnológicas adequadas às condições específicas de cada país, bem como um lineamento de políticas que promova e possibilite que tais infra-estruturas sejam incorporadas aos processos produtivos.

Nesse contexto, o Programa II visa a promover e apoiar as ações dos Estados membros destinadas a aprimorar a configuração de suas políticas tecnológicas, fortalecer a organização e administração de seus sistemas de geração e transferência de tecnologia e facilitar a transferência tecnológica internacional. Desse modo será possível fazer melhor aproveitamento de todos os recursos disponíveis e uma contribuição mais eficiente e efetiva para a solução dos problemas tecnológicos da produção agropecuária, num âmbito de igualdade na distribuição dos benefícios e de conservação dos recursos naturais.

INSTITUTO INTERAMERICANO DE COOPERAÇÃO PARA A AGRICULTURA

O Instituto Interamericano de Cooperação para a Agricultura (IICA) é o organismo especializado em agricultura do Sistema Interamericano. Suas origens datam de 7 outubro de 1942, quando o Conselho Diretor da União Pan-Americana aprovou a criação do Instituto Interamericano de Ciências Agrícolas.

Fundado como uma instituição de pesquisa agronômica e de ensino, de pós-graduação para os trópicos, o IICA, respondendo às mudanças e novas necessidades do Hemisfério, converteu-se progressivamente em um organismo de cooperação técnica e fortalecimento institucional no campo da agropecuária. Essas transformações foram reconhecidas oficialmente com a ratificação, em 8 de dezembro de 1980, de uma nova convênio, que estabeleceu como fins do IICA estimular, promover e apoiar os laços de cooperação entre seus 31 Estados membros para a obtenção do desenvolvimento agrícola e do bem-estar rural.

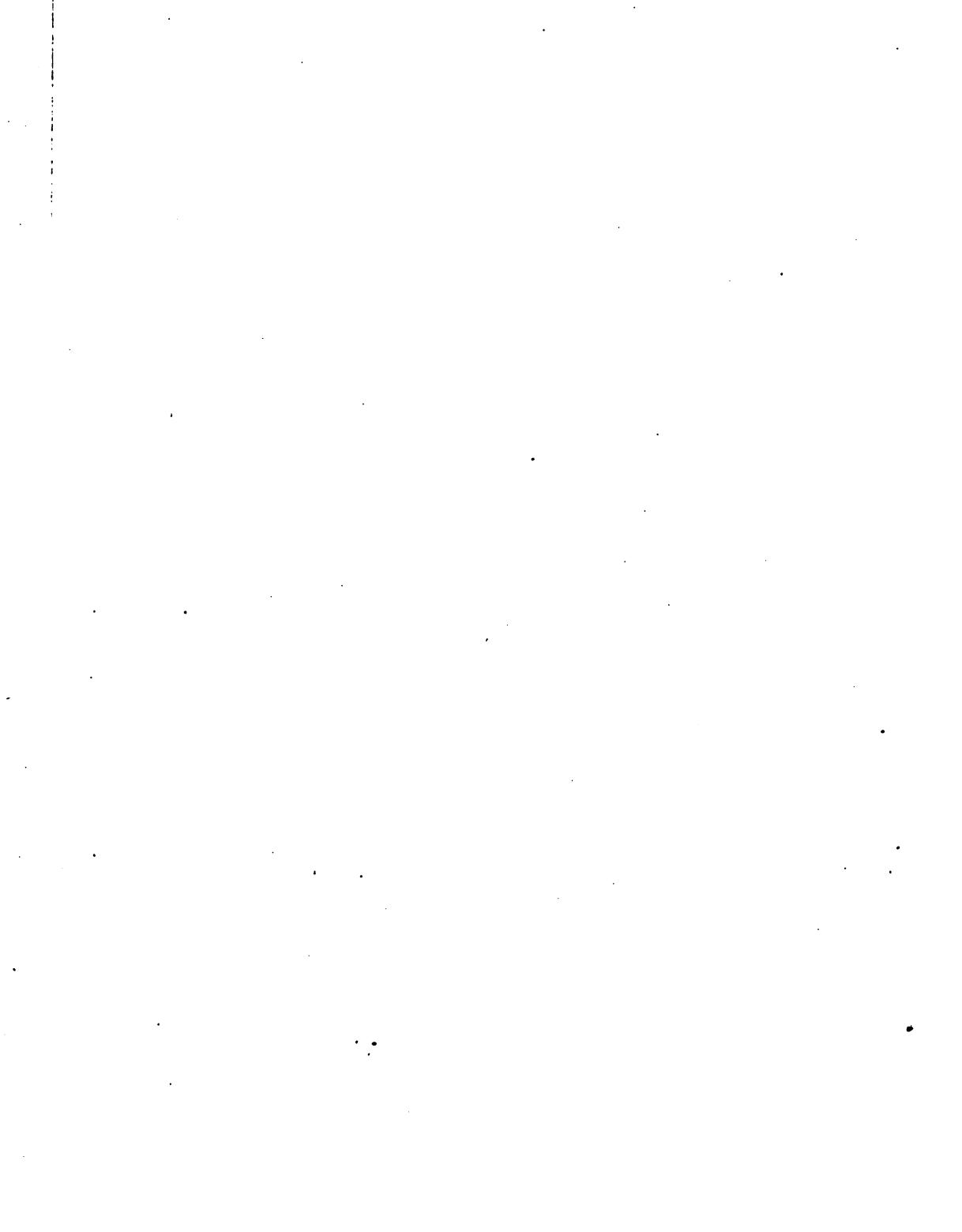
Com um mandato amplo e flexível e com uma estrutura que permite a participação direta dos Estados membros na Junta Interamericana de Agricultura e em seu Comitê Executivo, o IICA conta com ampla presença geográfica em todos os países membros para responder a suas necessidades e cooperação técnica.

As contribuições dos Estados membros e as relações que o IICA mantém com 12 Países Observadores, e com vários organismos internacionais, lhe permitem canalizar importantes recursos humanos e financeiros em prol do desenvolvimento agrícola do Hemisfério.

O Plano de Médio Prazo 1987-1991, documento normativo que assinala as prioridades do Instituto, enfatiza ações voltadas para a reativação do setor agropecuário como elemento central do crescimento econômico. Em vista disso, o Instituto atribui especial importância ao apoio e promoção de ações tendentes à modernização tecnológica do campo e ao fortalecimento dos processos de integração regional e sub-regional.

Para alcançar tais objetivos o IICA concentra suas atividades em cinco áreas fundamentais, a saber: Análise e Planejamento da Política Agrária; Geração e Transferência de Tecnologia; Organização e Administração para o Desenvolvimento Rural; Comercialização e Agroindústria, e Saúde Animal e Sanidade Vegetal.

Essas áreas de ação expressam, simultaneamente, as necessidades e prioridades determinadas pelos próprios Estados membros e o âmbito de trabalho em que o IICA concertra seus esforços e sua capacidade técnica, tanto sob o ponto de vista de seus recursos humanos e financeiros, como de sua relação com outros organismos internacionais.



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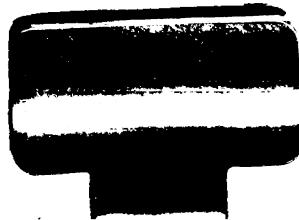
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