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Crystal Structure of the Ion Pair 1-Methyl-3-carbamidopyridinium *N*-Acetyl-L-tryptophanate, a Model for 1-Substituted Nicotinamide–Protein Charge-Transfer Complexes

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Abstract: The π–π^* charge-transfer complex, 1-methyl-3-carbamidopyridinium *N*-acetyl-L-tryptophanate, a model for charge-transfer complexes between 1-substituted nicotinamide derivatives and proteins, was investigated by the method of x-ray structure analysis. The phase problem was solved by direct methods and the structure was refined by standard methods (final $R = 0.057$). The yellow crystals are elongated in the $\vec{c}$ direction and the ion pairs form an extended alternating donor/acceptor stack in this direction, the planes of a donor/acceptor pair making an angle of 9° with each other. Within the stack, the ring–ring distances between a tryptophan donor and one of its neighbor acceptors are somewhat shorter than to the other neighbor. The donor/acceptor pair with these shorter distances also exhibits a larger degree of mutual ring–ring overlap, suggesting that discrete charge-transfer pairs may be favored within the stack. The hydrogen bond network links a given tryptophan moiety exclusively to the neighbor acceptor with the longer ring–ring distances. This network, combined with the attractive forces derived from the charge-transfer interaction, could explain the crystal habit. The calculated permanent dipole moments of the donor and acceptor are uncoupled in both the ground state and the excited state. The contribution of dipole–dipole interactions to the specificity of binding of NAD^+ to proteins is discussed.

The indole moiety of tryptophan forms a π_D–π_A^* charge-transfer complex with nicotinamide adenine dinucleotide (NAD^+) in model studies[2] and it has been proposed that this kind of complex is responsible for the long-wavelength absorption in NAD^+/3-phosphoglyceraldehyde dehydrogenase mixtures.[3] Similar complexes are formed between 1-substituted 3-carbamidopyridinium ions and various tryptophan derivatives as well as exposed indole groups in several proteins.[2a,4-8] The complexes of proteins with 1-methyl-3-carbamidopyridinium chloride (1-methylnicotinamide chloride) have been used extensively to study the environments of tryptophanyl and tyrosyl residues with regard to their solvent availability as a function of solvent composition.[4-9]

The nature of the surface interaction necessary for the formation of these π–π^* charge-transfer complexes is of some biological interest in that it may in part explain the specificity of binding of the coenzyme NAD^+ to various enzymes and provide a more detailed understanding of how the environment of a tryptophanyl residue affects the binding of nicotinamide analogues in solution. The interaction geometry of 1-(2-indol-3-ylethyl)-3-carbamidopyridinium chloride (I), an intramolecular model for these complexes, has been studied both in solution[10] and by the technique of x-ray crystallography.[11] These experiments indicated that in solution the compound adopted a folded gauche conformation while in the crystal the two ring systems were in an extended trans configuration. However, in the solid state the donor and acceptor moieties from adjacent molecules have a large amount of π-orbital overlap and alternate in an extended donor/acceptor stack in a manner similar to that of previously determined π-donor/π-acceptor complexes.[12] In the solid state, the calculated permanent dipole moments of the donor/acceptor pairs were apparently strongly coupled; this observation was subsequently used as part of a possible criterion for a stereospecific interaction between nicotinamide and tryptophan.

In an attempt to gain further insight into the potential energy minimum occupied by indole/nicotinamide charge-transfer complexes and the possible effects of crystal packing

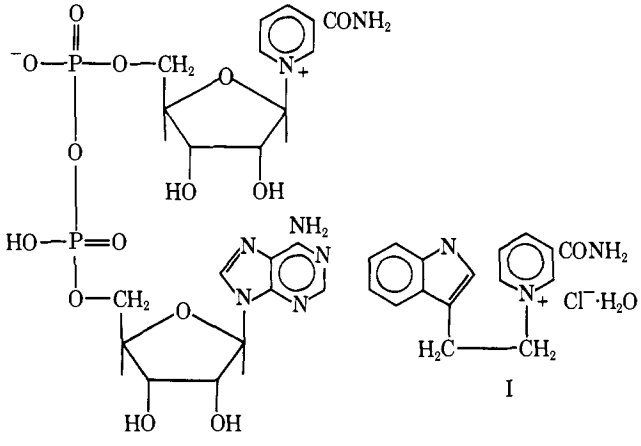

Table I. Final Atomic Parameters and Their Standard Deviations Given in Parentheses[a]

Atom	x	y	z	Atom	x	y	z
C-1	0.4737 (3)	0.0885 (1)	0.6451 (4)	H-11	0.4154 (32)	0.0619 (11)	0.6926 (40)
C-2	0.5988 (4)	0.0784 (1)	0.5671 (5)	H-21	0.6272 (32)	0.0448 (11)	0.5510 (41)
C-3	0.6873 (4)	0.1171 (2)	0.5128 (5)	H-31	0.7753 (33)	0.1095 (12)	0.4475 (43)
C-4	0.6509 (3)	0.1673 (1)	0.5321 (4)	H-41	0.7205 (37)	0.1932 (13)	0.4885 (48)
C-5	0.5241 (3)	0.1781 (1)	0.6105 (4)	H-81	0.2779 (29)	0.2421 (10)	0.7704 (38)
C-6	0.4347 (3)	0.1393 (1)	0.6714 (4)	H-91	0.1599 (30)	0.1634 (11)	0.9155 (37)
C-7	0.3173 (3)	0.1640 (1)	0.7462 (4)	H-92	0.2205 (27)	0.1069 (9)	0.8822 (32)
C-8	0.3401 (3)	0.2146 (1)	0.7279 (4)	H-101	−0.0027 (27)	0.1103 (9)	0.7740 (35)
C-9	0.1943 (3)	0.1396 (1)	0.8305 (4)	H-131	0.2099 (46)	0.0256 (16)	0.3779 (53)
C-10	0.0755 (3)	0.1261 (1)	0.7093 (4)	H-132	0.0739 (68)	−0.0009 (20)	0.3748 (74)
C-11	0.0162 (3)	0.1752 (1)	0.6315 (4)	H-133	0.1626 (45)	−0.0224 (16)	0.4784 (55)
C-12	0.0857 (4)	0.0408 (1)	0.5893 (5)	H-141	0.8390 (50)	0.0512 (15)	0.0815 (56)
C-13	0.1508 (5)	0.0088 (1)	0.4524 (6)	H-142	0.7646 (53)	0.0503 (18)	−0.1054 (66)
C-14	0.7389 (5)	0.0543 (2)	0.0148 (7)	H-143	0.6760 (38)	0.0302 (14)	0.0651 (46)
C-15	0.5477 (3)	0.1073 (1)	0.1192 (4)	H-151	0.5108 (26)	0.0771 (10)	0.1738 (32)
C-16	0.4825 (3)	0.1536 (1)	0.1409 (3)	H-171	0.5012 (26)	0.2304 (10)	0.0848 (34)
C-17	0.5412 (3)	0.1966 (1)	0.0683 (4)	H-181	0.7112 (33)	0.2218 (12)	−0.0833 (42)
C-18	0.6643 (3)	0.1922 (1)	−0.0211 (4)	H-191	0.8144 (34)	0.1433 (12)	−0.0930 (41)
C-19	0.7257 (3)	0.1457 (1)	−0.0374 (4)	H-211	0.4894 (29)	0.2587 (10)	0.6069 (36)
C-20	0.3485 (3)	0.1536 (1)	0.2362 (4)	H-221	0.1752 (32)	0.1013 (11)	0.4964 (40)
N-1	0.4633 (3)	0.2240 (1)	0.6494 (3)	H-241	0.3519 (36)	0.2303 (13)	0.2919 (46)
N-2	0.1206 (2)	0.0898 (1)	0.5836 (3)	H-242	0.2294 (29)	0.1974 (10)	0.3565 (35)
N-3	0.6681 (3)	0.1043 (1)	0.0323 (3)	H-351	−0.0025 (80)	0.1424 (27)	0.3010 (110)
N-4	0.3025 (3)	0.1980 (1)	0.2931 (4)	H-352	0.0720 (76)	0.1058 (27)	0.1876 (96)
O-1	−0.0438 (2)	0.2050 (1)	0.7281 (3)				
O-2	0.0365 (2)	0.1822 (1)	0.4751 (3)				
O-3	0.0105 (3)	0.0224 (1)	0.6970 (3)				
O-4	0.2858 (2)	0.1129 (1)	0.2562 (3)				
O-5	−0.0186 (4)	0.1188 (2)	0.1914 (4)				

[a] The atomic coordinates are given in fractions of a unit cell edge.

forces, the crystallographic structure of a compound in which the partners of a donor/acceptor pair were not restricted by any covalent bonds to adjacent donor/acceptor pairs was examined. The compound chosen was 1-methyl-3-carbamidopyridinium *N*-acetyl-L-tryptophanate (1-methylnicotinamide *N*-acetyltryptophanate, II), an ion pair.

Experimental Section

The charge-transfer salt was prepared by the reaction of equinormal amounts of 1-methylnicotinamide chloride, *N*-acetyl-L-tryptophan, and silver(I) oxide in an ethanol/water solution (50% v/v). After stirring for 2 h, the silver chloride precipitate was removed by filtration and the resulting solution evaporated to dryness under vacuum. The gummy yellow residue was further dried by repeatedly dissolving it in absolute ethanol, adding 4 vol of benzene, and evaporating to dryness under vacuum. This material, which probably still contained a small amount of water, was crystallized from a toluene/ethanol solution (25% v/v) to yield long, thin, yellow needles which were adequate for collection of x-ray data.

The density of these crystals was determined by flotation in a mixture of bromobenzene and *n*-heptane. Since perfect crystals of sufficient size were obtained only with some difficulty, of necessity the density determinations were performed on striated crystals which probably contained air. The average determination for two sets of crystals was 1.297 g/cm^3 as compared to a calculated density of 1.319 g/cm^3 based upon the contents of the unit cell plus one water of hydration per asymmetric unit. The difference between the two, 6.7 daltons per asymmetric unit, is greater than experimental error and is probably due to the imperfect nature of the crystals as mentioned above.

Preliminary space group and cell constant information was obtained from precession photographs; final orientation matrix and lattice parameter data were determined on a Picker FACS-1 four-circle diffractometer and refined by the method of least squares.

Crystal Data. Crystals of II ($C_7H_9N_2O^+C_{13}H_{13}N_2O_3^-\cdot H_2O$) are orthorhombic, space group $P2_12_12_1$, based on systematic absences in precession photographs and diffractometer data ($h00$) h odd, ($0k0$) k odd, and ($00l$) l odd; a = 9.639 (4) Å, b = 26.314 (9) Å, c = 7.952 (3) Å, V = 2017 Å^3; specimen size was 0.1 mm × 0.1 mm × 0.7 mm.

Intensity data were collected using Nb-filtered Mo Kα radiation employing the $\omega/2\theta$ scan technique. Of the 2661 unique reflections measured within the limiting sphere with a radius of $\sin\theta/\lambda = 0.7$, 1964 were at least two standard deviations (σ) above background, where:

$$\sigma = [N_{\text{pk scan}} + (T_{\text{scan}}/T_{\text{bkg}})^2 N_{\text{bkg}} + (0.01N_{\text{pk net}})^2]^{1/2}$$

Measurement of standards throughout data collection indicated that no deterioration correction was necessary. No absorption corrections were made.

Structure Solution. By assigning three origin defining phases and one enantiomorph defining phase, phases for 305 of the reflections with a normalized structure factor value greater than 1.5 were determined using the SIGMA and TANGEN programs of the 1970 version of the "X-ray System".[13] A Fourier synthesis using all 305 normalized structure factors revealed the position of 26 of the 29 heavy atoms in the asymmetric unit and indicated the approximate location of the remaining three; the R_E value at this point was 0.20, where:

$$R_E = \sum \| E_{\text{obsd}} | - | E_{\text{calcd}} \| / \sum | E_{\text{obsd}} |$$

E_{obsd} and E_{calcd} were the observed and calculated normalized structure factors, respectively. Subsequent difference syntheses and least-squares refinements refined the position of all 29 heavy atoms (R = 0.12). Atomic scattering factors for carbon, nitrogen, and oxygen were those of Berghuis et al.[14] and, for hydrogen, those of Stewart et al.[15] Two cycles of anisotropic refinement of the heavy atoms followed by a difference map located the 24 hydrogens (R = 0.087). After two subsequent cycles in which hydrogen atoms were refined isotropically and nonhydrogen atoms anisotropically, the final residual:

$$R = \sum \| F_o | - | F_c \| / \sum | F_o |$$

for the 2377 reflections,[16] including those reflections where F_c calculated larger than F_o, contributing to the Fourier sums was 0.057 and the weighted residual error was 0.047 where:

$$R = [\sum w(|F_o| - |F_c|)^2 / \sum w(F_o)^2]^{1/2}$$

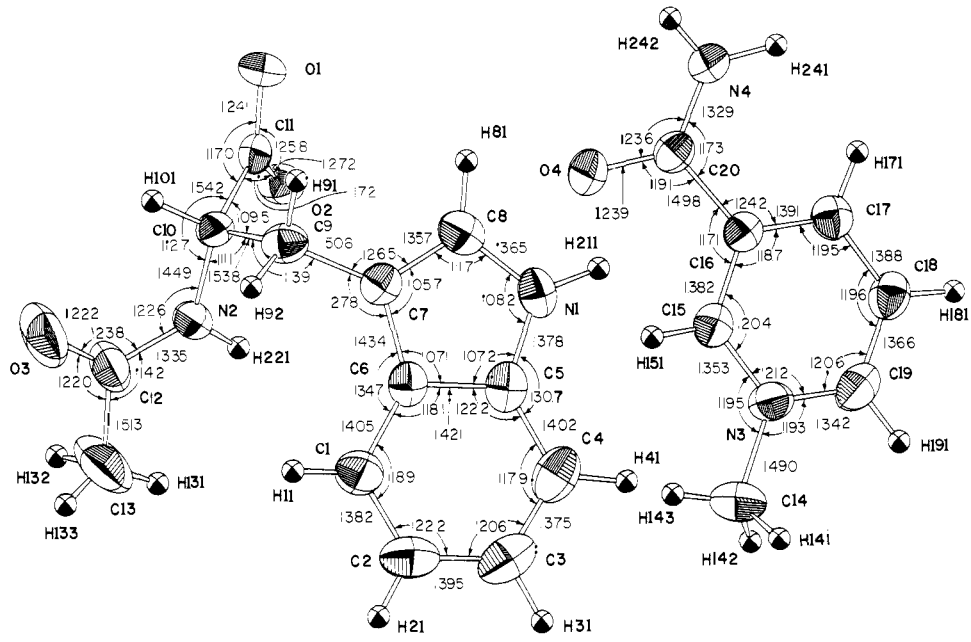

Figure 1. Bond lengths (in angstroms) and angles (in degrees) between nonhydrogen atoms of II. Standard deviations for the heavy atoms range from 0.004 to 0.006 Å in bond lengths and 0.2 to 0.3° in bond angles. Bond lengths have not been corrected for thermal motion. Nonhydrogen atoms are represented by thermal ellipsoids which have a 50% probability of containing the atom.

The standard deviation of an observation of unit weight:

$$\sigma = [\sum w(|F_o| - |F_c|)^2/(m-n)]^{1/2}$$

was 1.496. A three-dimensional difference synthesis revealed no peaks greater than 0.2 e/Å^3. Final atomic coordinates are listed in Table I.[16]

Results and Discussion

Bond lengths and angles determined for the heavy atoms of II are presented in Figure 1; the thermal ellipsoids are shown at a 50% probability level. Standard deviations for heavy atoms ranged from 0.004 to 0.006 Å in bond lengths and 0.2 to 0.3° in bond angles. Carbon–hydrogen and nitrogen–hydrogen distances were from 0.85 to 1.10 and from 0.86 to 1.00 Å, respectively. Thermal parameters were within the expected range although those for C-13, O-3, and O-5 were larger than the rest.

Least-squares planes were calculated for the indole ring system, the pyridine ring system, and the amide group of the nicotinamide moiety. The heavy atoms of the two ring systems were planar, the largest deviation from the least-squares planes being 0.02 Å; the angle between the indole and pyridine moieties was found to be 9°. This angle is relatively small, and allows for considerable π–π interaction between the donor and acceptor ring systems. Figure 2, a projection of an indole donor and two 1-methylnicotinamide acceptors onto the xz plane, shows some selected interatomic distances between the two ring systems. The distances between C-5 and C-18 and between C-8 and C-17 are somewhat short, indicating the possibility of some π–π interaction between the donor/acceptor pair.

The plane of the amide group of the 1-methylnicotinamide cation makes an angle of 18° with the plane of the pyridine ring. In the structure of I and in the structure of 1-methyl-3-carbamidopyridinium aden-9-yl acetate, III,[17] the two groups are nearly coplanar, having interplanar angles of 2.5 and 5.7°, respectively. Molecular orbital calculations predict that in the case of a reduced nicotinamide moiety the amide hydrogen will be straddled by the two ring hydrogens, and thus the two groups will be coplanar, while in the oxidized form the amide will make an angle of 30° with the pyridine ring.[18] These calculations have been borne out by x-ray study of several nicotinamide derivatives.[19-21] The structure of II, although not as

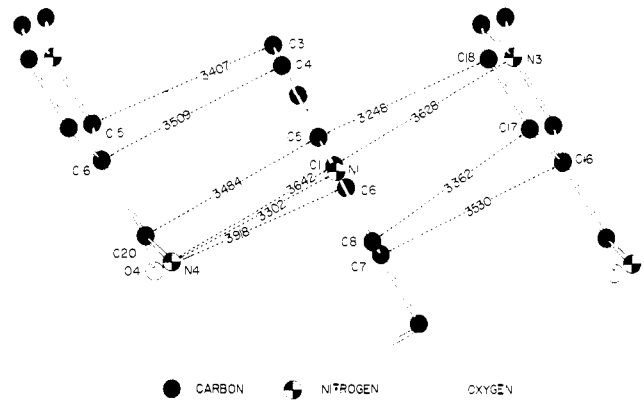

Figure 2. A partial view of a tryptophanyl donor and its two nearest neighbor 1-methylnicotinamide acceptors indicating selected interatomic distances between the two donor/acceptor pairs. The estimated standard deviations of these distances were not greater than 0.005 Å.

sterically hindered as those of I and III, is still displaced from the calculated potential energy minimum. This discrepancy, as well as those for I and III, may in part be counterbalanced by the two hydrogen bonds formed by the $-NH_2$ group in the nicotinamide of these compounds.

All hydrogens available to form hydrogen bonds were found to be within an appropriate distance and in an appropriate direction to form them with suitable electronegative acceptors. The donor–hydrogen–acceptor angles for five of the six hydrogen bonds ranged from 166.3 to 177.6°, while the angle for the last bond, that between O-4 and O-5, was 148.9°. This latter bond was also slightly long for an oxygen–oxygen bond, having a distance of 2.98 (3) Å between heavy atom centers. Figure 3 shows the hydrogen bonding scheme of an *N*-acetyltryptophan with its two nearest neighbor π acceptors, the view being perpendicular to the indole plane. It would appear that the network of bonds links only one of the nearest neighbor acceptors to a given donor in the donor/acceptor stacks formed in this structure. The structure of I, on the other hand, showed no hydrogen bonds to adjacent donors/acceptors, but instead formed bonds with the chloride counterion and the water of hydration found in the crystal. The crystals of II form needles

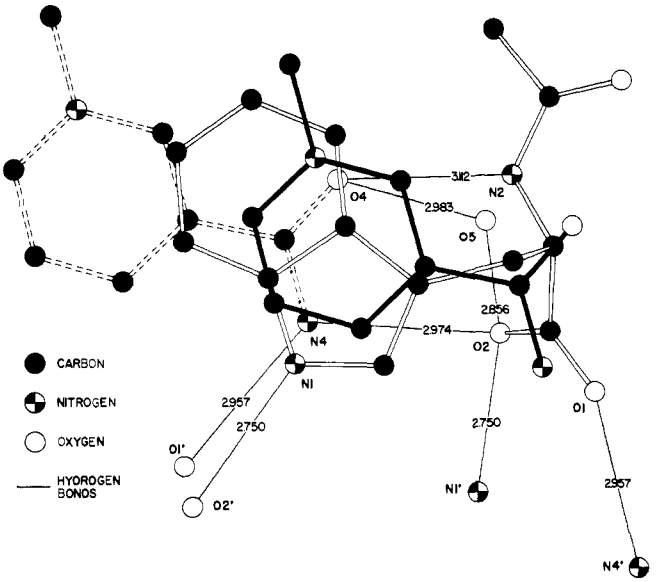

Figure 3. The hydrogen bonding scheme present in crystals of II. This figure, a view perpendicular to the indole plane, also illustrates the relative amounts of ring–ring overlap of the two nearest neighbor donor/acceptor pairs. The hydrogen bond network links a tryptophanyl donor exclusively with the nearest neighbor 1-methylnicotinamide having the smaller amount of ring–ring overlap, the same nicotinamide with the longer ring–ring distances in Figure 2. One of these links is through the water of hydration, O-5. N-1′, N-4′, O-1′, and O-2′ are related to the basic asymmetric unit by the screw axis parallel to the y axis and passing through the point 0.25,0,0.5 in fractions of a unit cell edge.

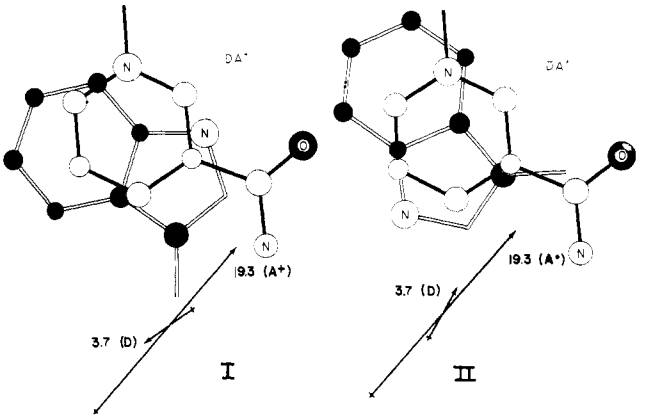

Figure 4. The approximate net charges on the atoms of the donor/acceptor pairs found in the ground state of the structures of I and II are depicted as projections of spheres having a volume proportional to the net charge. Atoms with a net negative charge are shaded while those with a net positive charge are open. The relative magnitude and direction of the calculated permanent dipole moments for the ground state of the donor and the ground state of the acceptor appear underneath the respective structures. (The largest net charge is about 0.4 e, on the nitrogens of the respective ring systems.)

elongated in the $\vec{c}$ direction; this is also the direction in which nicotinamide and indole ring systems form an extended stack. Figure 3 shows that one of the two nearest neighbor acceptors, the one not linked to a donor by hydrogen bonds, exhibits optimum overlap with the indole nucleus; this pair is also the one with the short ring–ring distances mentioned above. Although the association constants for charge-transfer complexes between indole derivatives and 1-methylnicotinamide are small,[5,8] this interaction coupled with the hydrogen bond network might explain the crystal habit. These data suggest that discrete charge-transfer pairs are favored within the stack.

The arrangement of the complex in the crystals is such that the calculated dipole moments for the donor and acceptor are almost completely uncoupled in both the ground state and the charge-transfer state. If one uses the position of the nicotinamide cation as a reference and compares structures I and II, the orientation of the indole has been rotated 180° about its long axis. Figure 4 depicts the overlap of the indole and nicotinamide ring systems of two nearest neighbors for the structures of I and II, the view being perpendicular to the indole plane. The heavy atoms are depicted as projections of spheres with the volumes proportional to their calculated charge in the ground state;[22] negatively charged atoms are shaded and the dipole moments are shown beneath the respective donor/acceptor pairs. Although the calculated permanent dipole moments of II are almost completely uncoupled, the two ring nitrogens, the most positively charged atoms of the system, are further removed from one another than in the structure of I. There appears to be more overlap between positively and negatively charged atoms in II than in I, while the positive–positive repulsion would appear about equal. The comparison of the charge states of I and II indicates the Coulombic attractions between specific atoms influence the exact interaction geometry in close contacts, while the dipole moment, an average quantity for a given molecule, may influence the orientation of a potential charge-transfer donor/acceptor pair as they approach one another. In the case of II, it would appear that the crystal packing forces have overcome the long-range effects of dipole–dipole coupling.

The structures of I and II when considered as models for other nicotinamide/indole charge-transfer complexes present two quite different views of the potential energy minimum occupied by these complexes and indicate caution in the extrapolation of these results to situations in which an acceptor such as NAD^+ would interact with an indole side chain of a protein. The dipole–dipole coupling of such a donor/acceptor pair might well have little effect in the relatively polar solvent in which the protein finds itself and certainly any of the other polar amino acids present nearby on the surface of the protein would perturb the local field.

On the other hand, in a suitable environment, such as the less polar conditions of the medium used to obtain crystals of II, one would expect that all Coulombic interactions would play a much stronger part in the determination of the final molecular arrangement of the complex. Likewise, one could imagine that if a nicotinamide derivative, such as NAD^+, approached a tryptophan residue in a relatively nonpolar cleft in a protein, a preliminary alignment due to dipole–dipole coupling in conjunction with increasing steric constraints would allow only one orientation of the molecule with the protein. The specificity of spatial arrangement, in this case perhaps the location of the chemically reactive part of the coenzyme, depends upon the assumption that only one surface of both the donor and acceptor is available to form a charge-transfer pair. Crystallographic evidence indicates that many tryptophan residues available to solvent have only one exposed surface and chemical evidence suggests that in NAD^+ one of the surfaces of the nicotinamide is covered by the adenine ring system.[23] Final association of the complex within the nonpolar environment of the cleft would depend upon the atom by atom interaction of the cofactor with the environment which would include all of the forces associated with van der Waals contacts, Coulombic interactions, hydrogen bonds, and the charge-transfer phenomenon, the same forces present in the crystal structure of model compounds.

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Supplementary Material Available: A listing of structure factor amplitudes, phases, and thermal parameters for individual atoms (4 pages). Ordering information is given on any current masthead page.

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Proton Transfer Reactions of Methylglyoxal Synthase

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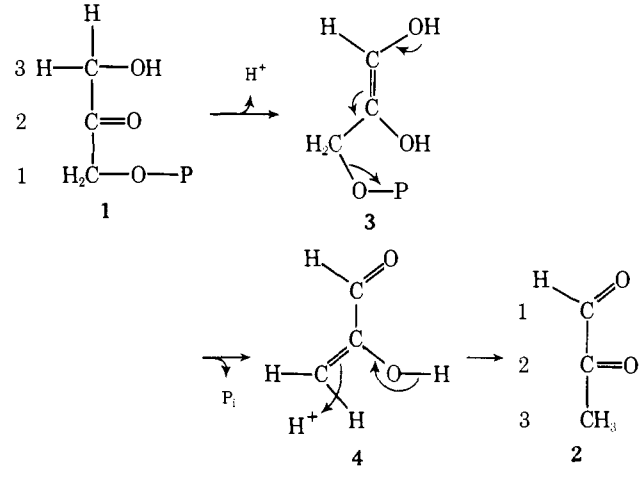
Abstract: *Escherichia coli* methylglyoxal synthase, which catalyzes the conversion of dihydroxyacetone-P to methylglyoxal, was shown to catalyze a stereospecific deprotonation of the *pro-S* hydrogen at C-3 of dihydroxyacetone-P (see Scheme II). Nonstereospecific protonation in the formation of the C-3 methyl group of methylglyoxal suggests that the true product of the enzymatic reaction is the enol form of methylglyoxal which is ketonized in solution. In agreement with this, methylglyoxal does not inhibit the enzyme.

Methylglyoxal synthase (also dihydroxyacetone-phosphate phospho-lase, EC 4.2.99.11) was discovered, purified, and studied in detail kinetically by Cooper and coworkers.[2-4] The enzyme, originally found to be apparently constitutive in *Escherichia coli* and other Enterobacteriaceae and also found in *Pseudomonas*, has recently been crystallized from *Proteus vulgaris*.[5] The role of this enzyme is not obvious since methylglyoxal is not known to have a function and may well be toxic. However, since the enzyme is very strongly inhibited by P_i it may be supposed that it serves to produce P_i from accumulated glycolytic stores in order that glyceraldehyde-3-P dehydrogenase can function.[3] With the well-known glyoxalase system, methylglyoxal synthase forms a bypass for the triose-P → lactate segment of the glycolytic path. Methylglyoxal synthase is specific for dihydroxyacetone-P and does not act on (*R,S*)-glyceraldehyde-3-P.[3] Under alkaline conditions dihydroxyacetone-P is known to produce methylglyoxal and P_i by way of an initial enolization followed by a 1,4 elimination (Scheme I).

Scheme I



If a similar reaction path is followed in the enzymic conversion of dihydroxyacetone-P to methylglyoxal it is of interest to ask the following questions. First, is the initial deprotonation to give the enediol intermediate **3** stereospecific? (The enediol of triose-P is believed to occur as an intermediate in both triose-P isomerase and metal-dependent aldolase reactions.) If so, what is the stereospecificity and does an isotope effect provide a suggestion of a rate-limiting step? Second, is there a stereochemical relationship between the first enolization step and formation of the methyl group of methylglyoxal which results from protonation of the intermediate **4**, i.e., is there a 1,3-proton transfer, and is formation of the methyl group stereospecific?

Experimental Section

E. coli methylglyoxal synthase was prepared from an ammonium sulfate fraction of glucose-grown cells available in this laboratory using steps similar to the procedure of Hopper and Cooper.[3] The enzyme