If the substitution in Hb Hijiyama were a neutral glutamine for a positive lysine residue, resulting also in two more negative charges per molecule, one would expect the two abnormal hemoglobins to have approximately the same electrophoretic mobility; in fact, Hb Hijiyama moves at about twice the speed of Hb Hofu. Thus the enhanced electrophoretic mobility of Hb Hijiyama over both Hb A and Hb Hofu can be explained on the basis of a substituent that contributes two negative charges per chain or four per molecule. This condition is fulfilled only if a negative glutamic acid replaces a positive lysine residue (*15*).

At least two other abnormal hemoglobins have been reported in which glutamic acid is substituted for lysine, and in which anodal migration at *p*H 8.6 is relatively rapid (*15*). In Hb I, the substitution is in the α-chain at the 16th residue; in Hb Nβ, in the β-chain at the 95th residue. Therefore Hb Hijiyama is yet another example to be added to the expanding list of abnormal hemoglobins. According to present convention, Hb Hijiyama is designated $\alpha_2\beta_2^{120\ \mathrm{Glu}}$.

The abnormality in our two cases seems to have no clinical effect, but individuals homozygous for the trait would offer convincing evidence in this respect. In view of the rarity of the gene, it is extremely unlikely that such individuals will be found, even in Japan with its somewhat elevated frequency of consanguinity.

The hemolysate of the proband contains 58 percent of the abnormal hemoglobins and stands at the top of the list of β-chain variants with respect to the relative proportion in the heterozygote's blood (*4*). The significance of this observation is obscure; it may indicate that Hb Hijiyama has a slightly greater affinity for heme than has Hb A, or that the protein-synthesis mechanisms for Hb Hijiyama are slightly more efficient than those for Hb A (*16*). We cannot yet choose between these and alternative explanations.

The trait is apparently mediated by a gene at the β-chain locus, allelic to the normal gene. Beale and Lehmann (*15*) noted that, according to the generally accepted concepts of the genetic code (*17*), single-base mutations in the triplet codons for amino acids in proteins account for all currently known hemoglobin variants. Hemoglobin Hijiyama is no exception. Thus, in messenger RNA, in which the code for lysine is either AAA or AAG, substitution of G in the first position produces GAA or GAG, either of which specifies glutamic acid (A, adenine; G, guanine).

TAKAOKI MIYAJI, YUZO OBA
KIYOMI YAMAMOTO, SUSUMU SHIBATA
Third Division of Internal Medicine, Yamaguchi University Faculty of Medicine, Ube City, Japan

IWAO IUCHI
Department of Clinical Pathology, Kawasaki Hospital, Okayama City, Japan

HOWARD B. HAMILTON
Department of Clinical Laboratories, Atomic Bomb Casualty Commission, Hijiyama, Hiroshima, Japan

References and Notes

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2. S. Shibata and I. Iuchi, *Acta Haematol. Japon.* **24**, 51 (1961).
3. We thank Grant Stemmermann, Kuakini Hospital, Honolulu, Hawaii, for obtaining these four blood samples.
4. S. Shibata, I. Iuchi, T. Miyaji, *Acta Haematol. Japon.* **29**, 115 (1966).
5. O. Smithies, *Biochem. J.* **61**, 629 (1955).
6. S. Ueda, *Japan. J. Clin. Hematol.* **3**, 26 (1962).
7. K. Singer, A. I. Chernoff, L. Singer, *Blood* **6**, 413 (1951); C. A. J. Goldberg, *Clin. Chem.* **4**, 146 (1958).
8. E. Bucci and C. Fronticelli, *J. Biol. Chem.* **240**, 551 (1962); T. Take, *J. Biochem.* **49**, 206 (1961).
9. S. Shibata, I. Iuchi, S. Ueda, T. Miyaji, I. Takeda, *Acta Haematol. Japon.* **25**, 675 (1962).
10. M. L. Anson and A. E. Mirsky, *J. Gen. Physiol.* **13**, 469 (1930); V. M. Ingram, *Biochim. Biophys. Acta* **28**, 539 (1958); C. Baglioni, *ibid.* **48**, 392 (1961); R. T. Jones, *Symp. Quant. Biol.* **29**, 297 (1964).
11. R. Archer and C. Crocker, in *Techniques in Protein Chemistry*, J. L. Bailey, Ed. (Elsevier, Amsterdam, 1962), pp. 21–2.
12. βTp, β tryptic peptide.
13. Fingerprints were lightly stained with 0.02 percent ninhydrin, the spots were cut out, and the peptides were eluted with 6*N* HCl; 22-hour hydrolysis at 105°C. Amino acid analysis was performed automatically with a Yanagimoto Automatic Amino Acid Analyzer, model LC-5, by the methods of S. Moore, D. H. Spackman, W. H. Stein, *Anal. Chem.* **30**, 1185 (1958); D. H. Spackman, W. H. Stein, S. Moore, *ibid.*, p. 1190.
14. G. Braunitzer, K. Hilse, V. Rudloff, N. Hilschmann, *Advan. Protein Chem.* **19**, 1 (1964).
15. D. Beale and H. Lehmann, *Nature* **207**, 259 (1965); R. B. Schneider, J. B. Alpern, D. Beale, H. Lehmann, *J. Lab. Clin. Med.* **68**, 940 (1966). Hb Nβ is also known as Hb N Baltimore or Hb N Memphis.
16. H. Lehmann and R. G. Huntsman, *Man's Hemoglobins* (North-Holland, Amsterdam, 1966), pp. 144–7.
17. M. Nirenberg, P. Leder, M. Bernfield, R. Brimacombe, J. Trupin, F. Rottman, C. O'Neal, *Proc. Nat. Acad. Sci. U. S.* **53**, 1161 (1965).
18. Aided by PHS research grant GM 09469-05. This report was presented in part before the 10th Annual Meeting of the Japanese Society of Human Genetics, Kumamoto City, Japan, 9–10 October 1965.

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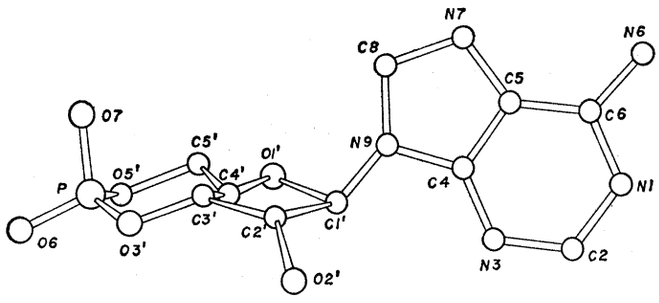
Crystal and Molecular Structure of Adenosine 3′,5′-Cyclic Phosphate

Abstract. *The structure of adenosine 3′,5′-cyclic phosphate has been determined by single-crystal x-ray diffraction. The two molecules in the asymmetric unit show different conformation about the glycosidic bond, while other structural details are essentially the same. The furanose rings are puckered with the C(4′) atom out of the best four-atom plane. The bond lengths and angles appear to be normal.*

Adenosine 3′,5′-cyclic phosphate (cyclic 3′,5′-AMP) has a role in many metabolic processes and can influence the activity of certain enzymes. For example, in tissues it is a factor in the conversion of inactive glycogen phosphorylase to the active form. Furthermore, cyclic 3′,5′-AMP stimulates the production or secretion of steroids and other hormones in some tissues.

Inasmuch as molecular configuration is probably related to biological activity, we have studied the configuration of the molecule and compared its conformational aspects with those of the related compounds, 3′-AMP (*1*) and 5′-AMP (*2*).

Crystals of cyclic 3′,5′-AMP were grown as fine needles by diffusion of xylene into an aqueous solution at *p*H 2. A small crystal of dimensions 0.3 by 0.1 by 0.03 mm was mounted for collecting photographic data. The orthorhombic unit cell has the following approximate dimensions: $a = 10.63$ Å, $b = 35.75$ Å, $c = 7.80$ Å. The only systematically absent reflections were $h00$, $0k0$ and $00l$ for odd h, k and l respectively. The space group was assumed, therefore, to be $P2_12_12_1$. Density of the crystals was measured by flotation in a solution of dichloromethane and tetrabromoethane and found to be 1.75 g cm^{-3}. This suggests that there are two molecules of cyclic 3′,5′-AMP per asymmetric unit, along with the equiv-



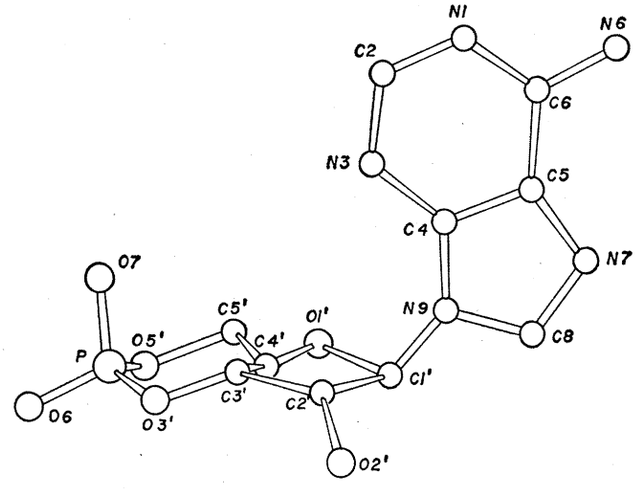


Fig. 1 (left). View of molecule I in the asymmetric unit.
Fig. 2 (right). View of molecule II showing different conformation.

alent of six or seven molecules of water.

The crystal from which the initial data were obtained was set aside for some months while an attempt was made to obtain larger crystals. After further efforts had produced none which were more suitable, work was resumed on the crystal used for the initial data. We were surprised to find that the b axis of the unit cell had decreased by about 16 percent, with a and c remaining virtually unchanged. The crystal was still single, although with a much increased mosaic spread. The unit cell parameters as determined from diffractometer measurements were as follows: $a = 10.65$ Å, $b = 29.71$ Å, $c = 7.80$ Å ($CuK\alpha = 1.5418$ Å), and the extinctions still indicated space group $P2_12_12_1$. If we assume the same density as previously, the crystal now corresponds to the anhydrous compound.

Intensities on the unidimensionally integrated photographs were measured with a recording microdensitometer having a logarithmic response. A Picker diffractometer equipped with a General Electric goniostat and a scintillation counter was used to collect 350 of the more intense reflections. In all, over 800 reflections were measured and used in the initial solution of the structure.

Interpretation of the three-dimensional Patterson function led to several possible positions for the P atoms, but there was no certainty that any combination of these was correct. A symbolic sign determination on the centric $hk0$ data led to several E-maps (*3*) for the projection on (001) containing possible P positions. All of these gave a vector for P—P corresponding to one of the interpretations of the Patterson function, and one of them gave P positions corresponding to prominent peaks in the Harker section, $P(u,v,1/2)$. Attempts to derive the z coordinates for the two P atoms led to 12 possible arrangements. These were tested in turn by Fourier synthesis based on the P positions. Once the correct combination had been determined, the location of the remaining atoms in the structure proceeded relatively smoothly using successive F_0 syntheses. The structure has been refined, thus far, to an R-index

$$(R \mid = \mid \Sigma \mid\mid F_o \mid - \mid F_c \mid\mid / \Sigma \mid F_o \mid)$$

of 0.13, with the use of isotropic thermal parameters.

Figures 1 and 2 show the two molecules in the asymmetric unit as viewed in a fixed direction with respect to the ribose rings. The molecule in Fig. 1 is oriented mainly along the a axis, with hydrogen bonding between N(6) of one molecule and O(3′) of the corresponding molecule in the adjacent unit cell. The second molecule, however, is oriented along the diagonal between the b and c axes. A relatively loose network of hydrogen bonds holds the crystal together.

The most important feature of the structure may be seen by comparing Figs. 1 and 2. The two molecules in the asymmetric unit show two quite different conformations about the glycosidic bonds. The differences may be expressed in terms of the torsion angle, ϕ_{CN}, which is defined by Donohue and Trueblood (*4*) as the angle formed between the trace of the plane of the base with the projection of the C(1′)—O(1′) bond when the projection is taken along the glycosidic bond. The torsion angle is taken as zero when O(1′) of the ribose and C(2) of the purine are antiparallel and the sense is positive for a clockwise rotation of the C(1′)—O(1′) projection relative to the trace of the base plane when viewed in the direction C(1′) to N(9). In molecule 1 this torsion angle is −50°, while it is +102° in molecule 2. Both are quite different from the values of −18° in adenosine-5′-phosphate (5′-AMP) and −4° in adenosine-3′-phosphate (3′-AMP). Steric effects between the ribose and the purine appear to affect the bond angles around N(9) equally in both conformations. The angle C(8)—N(9)—C(1′) in molecule 1 is 138°, while in molecule 2 the angle C(4)—N(9)—C(1′) is 137°.

The ribose ring in each molecule is puckered with C(4′) displaced by at least 0.6 Å from the best four-atom plane of the furanose ring. In 3′-AMP and 5′-AMP the C(3′) atom was displaced from the best plane, while some other nucleotides and nucleosides have shown puckering at the C(2′) position.

The phosphate groups appear to be quite normal with respect to bond angles, which range from 98° to 123°, and bond lengths in the range 1.44 Å and 1.68 Å. Figures 1 and 2 show that the rings containing the phosphorus atoms in both molecules are in a chair configuration.

KEITH WATENPAUGH
JEAN DOW, L. H. JENSEN
Department of Biological Structure, University of Washington, Seattle
SVEN FURBERG
Department of Chemistry, University of Oslo, Oslo, Norway

References and Notes

1. M. Sundaralingam, *Acta Cryst.* **21**, 495 (1966).
2. J. Kraut and L. H. Jensen, *ibid.* **16**, 79 (1963).
3. I. L. Karle, H. Hauptman, J. Karle, A. B. Wing, *ibid.* **11**, 257 (1958).
4. J. Donohue and K. N. Trueblood, *J. Mol. Biol.* **2**, 363 (1960).
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