Price, H., Kundu, S., and Ledeen, R. (1975), *Biochemistry 14,* 1512.

Race, R. R., and Sanger, R. (1968), Blood Groups in Man, 5th ed, Oxford, Blackwell Scientific Publications Ltd., p 136.

Saito, T., and Hakomori, S. (1971), *J. Lipid Res. 12,* 257.

Siddiqui, B., and Hakomori, S. (1973), *Biochim. Biophys. Acta 300,* 147.

Stellner, K., Saito, H., and Hakomori, S. (1973a), *Arch. Biochem. Biophys. 155,* 464.

Stellner, K., Watanabe, K., and Hakomori, S. (1973b), *Biochemistry 12,* 656.

Vance, D. E., and Sweeley, C. (1967), *J. Lipid Res. 8,* 621.

Watkins, W. M., and Morgan, W. T. J. (1964), *Proc. Congr. Int. Soc. Hematol., 9th, 1964,* 230.

Wherrett, J. R. (1973), *Biochim. Biophys. Acta 326,* 63.

Yamakawa, T., Nishimura, S., and Kamimura, M. (1965), *Jpn. J. Exp. Med. 35,* 201.

An Immunochemical Study of the Human Blood Group P_1, P, and P^k Glycosphingolipid Antigens†

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ABSTRACT: The erythrocyte P^k and P blood group antigens have been identified as ceramide trihexoside (CTH), Gal(α,1→4)Gal(β,1→4)Glc-Cer, and globoside, GalNAc(β,1→3)Gal(α,1→4)Gal(β,1→4)Glc-Cer, respectively, and the following structure has been proposed for the P_1 antigen: Gal(α,1→4)Gal(β,1→4)GlcNAc(β,1→3)Gal(β,1→4)Glc-Cer. Although the P_1 and P^k determinants have identical terminal disaccharides, CTH did not inhibit anti-P_1. The P_1 glycolipid and hydatid cyst glycoprotein inhibited the agglutination of $P_1{}^k$ erythrocytes by anti-P_1 and unabsorbed anti-P_1PP^k sera, but neither antigen inhibited a specific anti-P^k serum. The P_1 and P^k glycolipids were equally effective in inhibiting the hemagglutinating activity of a lectin with α-galactosyl specificity obtained from ova of *Salmo trutta.* Anti-P sera were inhibited most effectively by human erythrocyte globoside, and to a lesser extent by Forssman glycolipid and rat kidney globoside. In the latter glycolipid the linkage between the internal galactosyl residues is α,1→3, rather than α,1→4, as in erythrocyte globoside. No cross-reactions between P and P_1 or P^k antigens were detected. New hypotheses are offered to explain the genetic regulation and biosynthesis of the P_1, P, and P^k antigens.

The human P blood group system consists of three antigens, P_1, P, and P^k (Table I) (Race and Sanger, 1968). These antigens were demonstrated originally on erythrocytes, but they have been detected also on skin fibroblasts and lymphocytes (Gurner and Coombs, 1958; Fellous et al., 1973, 1974). P^k is unusual among blood group antigens in not being expressed as a codominant character (Matson et al., 1959; Kortekangas et al., 1965), i.e. the P^k antigen is detectable only on the erythrocytes of homozygous P^kP^k individuals and not in P^k heterozygotes.

Immunochemical studies of a cross-reacting glycoprotein obtained from hydatid cyst fluid (Cameron and Staveley, 1957) demonstrated that both P_1 and P^k antigens have carbohydrate determinants with an immunodominant terminal nonreducing α-galactosyl residue (Watkins and Morgan, 1964; Voak et al., 1973). There is some cross-reactivity between the P_1 and P^k antigens (Voak et al., 1973) but neither antigen cross-reacts with P. The genetic and biosynthetic relationships among these antigens are unclear, but it was suggested recently (Fellous et al., 1974) that P is the precursor of the P^k antigen.

We recently reported the identification of the P and P^k antigens as the glycosphingolipids globoside and ceramide trihexoside (CTH), respectively (Naiki and Marcus, 1974). We now present more comprehensive data on the immunochemistry of the P_1, P, and P^k antigens, and offer a hypothesis concerning their genetic regulation and biosynthesis.

Materials and Methods

Glycosphingolipids (Table II). Globosides isolated from bovine adrenal glands (Kawanami, 1967) and rat kidney (Kawanami and Tsuji, 1968; Siddiqui et al., 1972) were obtained from Dr. J. Kawanami (Shionogi Research Lab., Japan). Asialo G_{M2} was prepared by mild acid hydrolysis of G_{M1} and chromatography on a column of silicic acid (Naiki et al., 1974). The sources of the other glycolipids were described in the preceding paper (Naiki et al., 1975).

Erythrocytes and Antisera. Group O,P_1, and O,P_2 cells were obtained from normal donors. Samples of O,p and $O,P_1{}^k$ erythrocytes were obtained from Mr. W. L. Marsh of the New York Blood Center. Anti-P_1 was obtained from an O,P_2 donor; anti-P sera from individuals of the P_1^k phenotype were gifts from Dr. P. Tippett (Blood Group Reference Laboratory, London), Dr. D. J. Anstee (South West Regional Blood Transfusion Centre, Bristol), and Mr. W. L. Marsh. Three anti-P_1PP^k sera from p individuals were gifts from Dr. P. Levine (Ortho Research Foundation, Raritan, N.J.). These antisera have a complex specificity and agglutinate erythrocytes of all phenotypes except p. Different as-

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Table I: The P Blood Group System.

Phenotype	Frequency	Antigens on Erythrocytes	Antibodies in Serum
P_1	75%	P_1, P	None
P_2	25%	P	Anti-P_1
p	Very rare	None	Anti-P_1PP^k [a]
P^k_1	Very rare	P_1, P^k	Anti-P
P^k_2	Very rare	P^k	Anti-P

[a] These sera agglutinate erythrocytes of all phenotypes except p, but they do not necessarily contain three separable nonspecific antibodies, i.e., specific anti-P^k reagents can be made by absorption of some sera with P_1 erythrocytes but anti-P^k and anti-P_1 antibodies are totally removed from other sera by this procedure.

pects of their specificity can be examined by using test erythrocytes that lack some P antigens, e.g., P_2 cells lack P_1 and P^k antigens and react only with the anti-P, and P^k_1 cells possess P_1 and P^k but lack P antigen. An extract of *Salmo trutta* ova containing a lectin with α-galactosyl specificity (Anstee et al., 1973) was a gift from Dr. D. Voak.

Hemagglutination. All tests were carried out as described in the previous paper (Naiki et al., 1975) with the additional feature that ficin-treated erythrocytes were used in all assays except anti-P_1. A 4% suspension of erythrocytes was incubated in PBS[1] containing 1 mg/ml of crude ficin (Worthington Biochemical Corp.) for 15 min at 37°C, washed three times in PBS, and made up to a final concentration of 2%.

Results

P_1 and P^k Specificities. Agglutination of P_1 erythrocytes by anti-P_1 was inhibited only by hydatid cyst glycoprotein and the P_1 glycolipid (Table III). As indicated in the previous paper (Naiki et al., 1975) treatment of this glycolipid with α-galactosidase completely destroyed its P_1 activity. Although the terminal nonreducing disaccharides of the P_1 glycolipid and CTH are identical, CTH did not inhibit anti-P_1 in the concentration range employed.

Agglutination of P^k_1 erythrocytes by the four anti-P_1PP^k sera was inhibited by CTH, the P_1 glycolipid, and hydatid cyst glycoprotein (Table III). A specific anti-P^k serum was obtained by absorbing Haut serum with B,P_1 erythrocytes (Table IV), which removed anti-P_1 antibodies and those anti-P^k antibodies that cross-react with P_1. CTH was the only substance that inhibited the anti-P^k serum, but only small quantities of P_1 glycolipid were available for this experiment. Most of the antibodies in the four anti-P_1PP^k sera that react with P^k_1 erythrocytes are directed against the P^k determinant, as indicated by the titers of these sera with P_1 and P^k_1 erythrocytes (Table IV), and by the inhibition data.

Extracts of mature ova of *Salmo trutta* contain a hemagglutinin with α-galactosyl specificity (Anstee et al., 1973) that agglutinates human erythrocytes of all phenotypes except A_1,p and O,p. Agglutination of P_2 erythrocytes by this extract was completely inhibited by 1.5 μg/ml of CTH or 1.3 μg/ml of P_1 glycolipid, but not by any of the other glycolipids listed in Table II and a number of other glycolipids.

[1]Abbreviations used are: Gal, D-galactose; Glc, D-glucose; GalNAc, *N*-acetyl-D-galactosamine; GlcNAc, *N*-acetyl-D-glucosamine; NAN, *N*-acetylneuraminic acid; ceramide (Cer), *N*-acylsphingosine; PBS, 0.02 *M* potassium phosphate (pH 7.3)–0.15 *M* NaCl; CTH, ceramide trihexoside.

The extract also gave a precipitin band in agarose gel with CTH–sodium taurocholate (0.25 mg/ml CTH, 1.25 mg/ml taurocholate), but not with sodium taurocholate alone, or taurocholate mixed with globoside, paragloboside, or other glycolipids.

P Specificity. A similar pattern of inhibition was obtained with three anti-P and four anti-P_1PP^k sera (Table V). The best inhibitors were globosides from human erythrocytes or bovine adrenal glands, which have identical oligosaccharide chains. Rat kidney globoside, which differs from the other two in the position of the linkage between the two galactosyl residues (Table II), was a less effective inhibitor, and Forssman glycolipid was the least effective.

Glycosphingolipids of $P_1{}^k$ and p Erythrocytes. Globoside is the most abundant glycolipid of human erythrocytes, and CTH is one of the major neutral glycolipids (Vance and Sweeley, 1967; Ando and Yamakawa, 1973). Our immunological analysis of the P system suggested that P^k_1 erythrocytes, which lack P antigen, should have very little globoside and increased quantities of CTH, and p erythrocytes should be deficient in both globoside and CTH. We have recently analyzed the neutral glycolipids from erythrocytes of two P^k_1 and two p individuals and found that P^k_1 erythrocytes had no detectable globoside and a striking increase in CTH, and p erythrocytes had no detectable CTH or globoside (Marcus and Naiki, 1975). These results will be reported in detail elsewhere.

Discussion

The postulated structures of the P_1 and P^k antigens are consistent with previous data on the presence of an immunodominant terminal nonreducing α-galactosyl residue in both determinants, and with their cross-reactivity. The relatively restricted cross-reactivity between CTH and P_1 glycolipids is surprising because they have identical terminal nonreducing disaccharides. The two terminal disaccharides may differ significantly in their preferred conformations, however, because of interactions with adjacent sugar residues and/or the ceramide portion of the molecule. A precise analysis of the immunological relationship between these two glycolipids was hindered by the very limited quantity of P_1 glycolipid available, and also by the nature of the human antibodies employed. Three different types of anti-P_1 and anti-P^k specificities can be identified in human sera: specific anti-P_1 antibodies present in sera of P_2 individuals; antibodies in anti-P_1PP^k sera that cross-react with P_1 and P^k determinants; and specific anti-P^k antibodies that remain after absorption of these sera with P_1 cells. These "naturally occurring" antibodies were presumably produced against cross-reactive materials in the environment (Roland, 1973), as in the ABH system, and the relationship between these two antigens should be studied with antisera prepared against purified glycolipids. We have prepared antibodies to CTH and plan to make antibody to the P_1 glycolipid.

Globoside was first identified as the P antigen on the basis of hemagglutination inhibition data (Naiki and Marcus, 1974). Its absence in P^k_1 and p cells, which lack P antigen, provides conclusive evidence for this designation. The cross-reaction of Forssman glycolipid with anti-P sera is consistent with the weak reciprocal cross-reactions observed between rabbit antisera to the two glycolipids (Ishibashi et al., 1974; Laine et al., 1974). The α,1→3 linkage between the terminal and subterminal GalNAc residues of Forssman apparently allows some antibodies to bind to the globoside structure which is contained within the Forssman pentasac-

Table II: The Structures of Glycosphingolipids Used in These Experiments.[a]

Ceramide dihexoside (CDH)	Gal(β,1→4)Glc-Cer
Ceramide trihexoside (CTH) (P^k)	Gal(α,1→4)Gal(β,1→4)Glc-Cer
Globoside-human red cells (P)	GalNAc(β,1→3)Gal(α,1→4)Gal(β,1→4)Glc-Cer
Globoside-rat kidney	GalNAc(β,1→3)Gal(α,1→3)Gal(β,1→4)Glc-Cer
P_1 antigen	Gal(α,1→4)Gal(β,1→4)GlcNAc(β,1→3)Gal(β,1→4)Glc-Cer
Paragloboside	Gal(β,1→4)GlcNAc(β,1→3)Gal(β,1→4)Glc-Cer
Ganglioside	NAN(α,2→3)Gal(β,1→4)GlcNAc(β,1→3)Gal(β,1→4)Glc-Cer
Forssman	GalNAc(α,1→3)GalNAc(β,1→3)Gal(α,1→4)Gal(β,1→4)Glc-Cer
Asialo G_{M2}	GalNAc(β,1→4)Gal(β,1→4)Glc-Cer

[a] Abbreviations used are given in footnote 1.

Table III: Hemagglutination Studies with Anti-P_1 and Anti-P^k Antisera.[a]

		Lowest Concn of Antigen (μg/ml) That Completely Inhibits Hemagglutination					
		P_1 Erythrocytes,	P_1^k Erythrocytes				
			Anti-P_1PP^k				Anti-P^k, Absorbed
Inhibitor	Highest Concn Used for Test (μg/ml)	Anti-P_1	Im.	Ha.	Lu.	Haut	Haut[b]
Hydatid cyst glycoprotein	50	0.62	6.2	–[c]	6.2	25	–
P_1 glycolipid	5	0.04	0.62	1.2	1.2	5	–
CTH	50	–	3.1	1.5	0.4	3.1	6.2

[a] None of these sera was inhibited by 50 μg/ml of globoside, paragloboside, or Forssman glycolipids. [b] Haut serum absorbed with B,P_1 erythrocytes. [c] Indicates no inhibition.

Table IV: Specificity of Anti-P_1PP^k Sera.[a]

	Erythrocytes		
Antisera	O,P_1^k	O,P_1	O,P_2
Im	1:32	1:4	1:64
Ha	1:64	1:2	1:16
Lu	1:256	1:4	1:64
Haut	1:256	1:4	1:128
Absorbed Haut	1:128	–[b]	–

[a] Anti-P^k and anti-P titers were determined with O,P_1^k cells and O,P_2 cells, respectively. Anti-P_1 titers were determined with O,P_1 cells after absorption of the sera with 0.25 vol of O,P_2 packed cells at 4°C for 2 hr. [b] Negative at 1:2 dilution.

Table V: Hemagglutination Inhibition Studies with Anti-P Sera.[a]

		Lowest Concn of Antigen That Completely Inhibits Hemagglutination of P_2 Erythrocytes						
		Anti-P			Anti-P_1PP^k			
Inhibitor	Highest Concn Used for Test (μg/ml)	Ni.	Es.	Br.	Im.	Ha.	Lu.	Haut
Globoside-RBC	50	3.1	1.5	6.2	12.5	3.1	6.2	3.1
Globoside-adrenal gland	50	3.1	0.7	6.2	6.2	3.1	6.2	3.1
Globoside-rat kidney	50	6.1	1.5	25	12.5	3.1	25	6.2
Forssman	50	12.5	3.1	–[b]	12.5	25	50	12.5

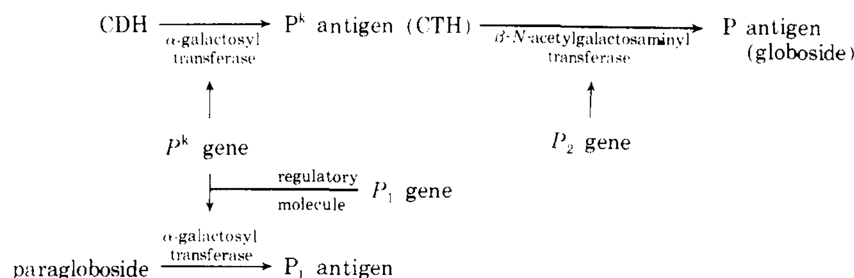
[a] None of these sera was inhibited by 5 μg/ml of P_1 glycolipid, 100 μg/ml of asialo G_{M2}, or 50 μg/ml of CTH, paragloboside, or hydatid cyst glycoprotein. [b] Indicates no inhibition.

charide. Some human "anti-P" may be, in fact, antibodies to Forssman antigen which is ubiquitous in the environment (Springer, 1971).

The suggestion that P^k is inherited in a recessive manner arose from the observation that it was not detectable on the erythrocytes of obligate heterozygotes, i.e., parents or children of P^k persons. P^k (CTH) is actually present in all erythrocytes expect p, but is not detected by the usual immunological criteria of agglutination or capacity to absorb anti-P^k antibodies. CTH concentration is much lower in normal erythrocytes than in P^k cells (Marcus and Naiki, 1975) and if glycolipids are clustered in the cell membrane the CTH trisaccharide may be sterically obstructed by the longer, more abundant globoside tetrasaccharides. The lack of a direct correlation between the concentration of a glycolipid in a cell membrane and its reactivity with antibody suggests that the organization and topography of the membrane are important. For example, baby hamster kidney cells (BHK) transformed by oncogenic viruses contain much less Forssman glycolipid than normal BHK cells, but the transformed cells react much more strongly with anti-Forssman antibodies (Hakomori, 1973). Despite the extremely high concentration of globoside in adult human erythrocytes (about 9 mg/100 ml of packed cells), these cells are barely agglutinated by anti-globoside antibodies, but agglutination is produced by a very high dilution of anti-globoside antibodies after treatment of the erythrocytes with proteolytic enzymes (Hakomori, 1973). In contrast, untreated erythrocytes obtained from umbilical cord blood are readily agglutinated by anti-globoside and no increase in titer is obtained by treatment of these cells with proteolytic enzymes.

Elucidation of the structures of the three P antigens provides a basis for considering their biosynthetic pathways and genetic regulation. The primary product of the P_2 gene is probably a β-*N*-acetylgalactosaminyltransferase that con-

Scheme I



verts CTH to globoside. The P_1^k phenotype, elevation of CTH levels in the erythrocyte and the absence of globoside, probably represent defective biosynthesis of globoside, although excessive degradation of globoside by a galactosaminidase could produce the same changes in glycolipid concentrations.

The synthesis of the P_1 and P^k determinants presents a more complex problem. The presence of identical terminal disaccharides in the P_1 and P^k determinants raises the possibility that a single α-galactosyltransferase may add the terminal sugar to both antigens. This scheme provides an economical explanation for the p phenotype in which all three antigens are missing: a primary deficiency in α-galactosyltransferase activity would result in loss of the P_1 and P^k antigens, and the P_2 gene would not be expressed for lack of a substrate. Postulation of a single transferase presents another problem, however: why do P_2 individuals synthesize CTH (the precursor of globoside) but lack detectable P_1 antigen? There are several possible explanations for this apparent discrepancy. (1) Paragloboside, the precursor of P_1 glycolipid, may not be available in sufficient quantity. This could result either from a defect in synthesis, or from preferential utilization of paragloboside in another biosynthetic pathway, such as the synthesis of the ganglioside that contains *N*-acetylglucosamine. Our preliminary analyses of the erythrocyte glycolipids of P_2 individuals indicate that this is not likely. (2) The α-galactosyltransferase synthesized by P_2 individuals might utilize only CDH as a substrate. The P_1 gene might produce a regulatory molecule that alters the acceptor specificity of the α-galactosyltransferase to enable it to utilize paragloboside as a substrate in addition to CDH (Scheme I). Regulation of the acceptor specificity of mammary gland β-galactosyltransferase by α-lactalbumin (Brodbeck et al., 1967; Brew et al., 1968) provides a model for this hypothesis. (3) The P_1 antigen may be synthesized but its immunological reactivity is blocked by the addition of sugars such as fucose or sialic acid to the P_1 determinant.

An alternative hypothesis is that the P_1 and P^k determinants are synthesized by different α-galactosyltransferases that are products of the P_1 and P^k genes. Individuals of the P_2 phenotype would lack the P_1 transferase that utilizes paragloboside as an acceptor. Individuals of the p phenotype would lack both α-galactosyltransferases, and since the probability of two independent mutations occurring in the same individual is small, one would have to postulate that the P_1 and P^k genes are closely linked and are both affected by a single genetic lesion. Regardless of whether there are one or two α-galactosyltransferases, it is unlikely that the P_1 and P_2 genes are alleles, as suggested previously (Race and Sanger, 1968). This hypothesis (Sanger, 1955) was advanced to explain the simultaneous loss of P_1 and P antigens in the p phenotype (originally designated Tj(a−) (Levine et al., 1951), and the relationship between P_1 and P antigens was thought to be similar to A_1 and A_2 antigens of the ABO blood group system. It is now clear that this analogy is not valid. Although the nature of the chemical difference between the A_1 and A_2 antigens is not established, both determinants have terminal nonreducing α-*N*-acetylgalactosaminyl residues and they are cross-reactive (Moreno et al., 1971). In contrast, the P_1 determinant has a terminal α-galactosyl residue, P has a terminal β-*N*-acetylgalactosaminyl residue, and these antigens are not cross-reactive. The recent proposal (Fellous et al., 1974) that the P antigen is a biosynthetic precursor of P^k is not tenable in view of the immunochemical data presented in these two papers.

The biosynthetic scheme proposed above can be tested by characterizing the transferases involved in the synthesis of these three glycolipids. Globoside and CTH are widely distributed in human tissues and the P_1 antigen has been detected recently in blood lymphocytes and skin fibroblasts (Fellous et al., 1974), so there are a number of potential sources for these enzymes. The transferases may even be present in plasma, like the enzymes of the ABH system (Kim et al., 1971; Sawicka, 1971; Schachter et al., 1971).

References

Ando, S., and Yamakawa, T. (1973), *J. Biochem. (Tokyo) 73,* 387.

Anstee, D. J., Holt, P. D. J., and Pardoe, G. (1973), *Vox Sang. 52,* 347.

Brew, K., Vanaman, T. C., and Hill, R. L. (1968), *Proc. Natl. Acad. Sci. U.S.A. 50,* 491.

Brodbeck, U., Denton, W. L., Tanahashi, N., and Ebner, I. E. (1967), *J. Biol. Chem. 242,* 1391.

Cameron, G. L., and Staveley, J. M. (1957), *Nature (London) 179,* 147.

Fellous, M., Couillin, P., Neauport-Sautes, C., Frezal, J., Bilardon, C., and Dausset, J. (1973), *Eur. J. Immunol. 3,* 543.

Fellous, M., Gerbal, A., Tessier, C., Frezal, J., Dausset, J., and Salmon, C. (1974), *Vox Sang. 26,* 518.

Gurner, B. W., and Coombs, R. R. A. (1958), *Vox Sang. 3,* 13.

Hakomori, S. (1973), *Adv. Cancer Res. 18,* 265.

Hakomori, S., Kijimoto, S., and Siddiqui, B. (1972), *Membr. Res., ICN-UCLA Symp. Mol. Biol., Proc., 1st,* 253.

Ishibashi, T., Kijimoto, S., and Makita, A. (1974), *Biochim. Biophys. Acta 337,* 92.

Kawanami, J. (1967), *J. Biochem. (Tokyo) 62,* 205.

Kawanami, J., and Tsuji, T. (1968), *Jpn. J. Exp. Med. 38,* 11.

Kim, Y. S., Perdomo, J., Bella, A., and Nordberg, J. (1971), *Proc. Natl. Acad. Sci. U.S.A. 68,* 1753.

Kortekangas, A. E., Kaarsalo, E., Melartin, L., Tippett, P., Gavin, J., Noades, J., Sanger, R., and Race, R. R.

(1965), *Vox Sang. 10,* 385.
Laine, R. A., Yogeeswaran, G., and Hakomori, S. (1974), *J. Biol. Chem. 249,* 4460.
Levine, P., Bobbitt, O. B., Waller, R. K., and Kuhmichel, A. (1951), *Proc. Soc. Exp. Biol. Med. 77,* 403.
Marcus, D. M., Bastani, A., Rosenfield, R. E., and Grollman, A. P. (1967), *Transfusion 7,* 277.
Marcus, D. M., and Naiki, M. (1975), manuscript in preparation.
Martensson, E. (1969), *Prog. Chem. Fats Other Lipids 10,* Part 4.
Matson, G. A., Swanson, J., Noades, J., Sanger, R., and Race, R. R. (1959), *Am. J. Hum. Genet. 11,* 26.
Moreno, C., Lundblad, A., and Kabat, E. A. (1971), *J. Exp. Med. 134,* 439.
Naiki, M., Fong, J., Ledeen, R., and Marcus, D. M. (1975), *Biochemistry,* preceding paper in this issue.
Naiki, M., and Marcus, D. M. (1974), *Biochem. Biophys. Res. Commun. 60,* 1105.
Naiki, M., Marcus, D. M., and Ledeen, R. (1974), *J. Immunol. 113,* 84.
Race, R. R., and Sanger, R. (1968), Blood Groups in Man, Oxford, 5th ed, Blackwell Scientific Publications Ltd., p 136.
Roland, F. (1973), *Ann. Microbiol. (Paris)* 124A, 375.
Sanger, R. (1955), *Nature (London) 176,* 1163.
Sawicka, T. (1971), *FEBS Lett. 16,* 346.
Schachter, H., Michaels, M. A., Crookston, M. C., Tilley, C. A., and Crookston, J. H. (1971), *Biochem. Biophys. Res. Commun. 45,* 1011.
Siddiqui, B., Kawanami, J., Li, Y., and Hakomori, S. (1972), *J. Lipid Res. 13,* 657.
Springer, G. F. (1971), *Prog. Allergy 15,* 9.
Vance, D. E., and Sweeley, C. (1967), *J. Lipid Res. 8,* 621.
Voak, D., Anstee, D., and Pardoe, G. (1973), *Vox Sang. 25,* 263.
Watkins, W. M., and Morgan, W. T. J. (1964), *Proc. Congr. Int. Soc. Hematol., 9th, 1964,* 230.

Proton Magnetic Resonance Studies of Double Helical Oligonucleotides. The Effect of Base Sequence on the Stability of Deoxydinucleotide Dimers†

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ABSTRACT: The concentration dependence of the proton magnetic resonance chemical shifts of a series of deoxydinucleotides and deoxydinucleoside monophosphates in neutral H_2O solution has been recorded in the 1-100 m*M* concentration range by the use of pulsed Fourier transform techniques. The self-complementary molecules pdG-dC, dG-dC, pdC-dG, and dC-dG and the complementary mixtures pdG-dG + pdC-dC as well as pdG-dT + pdA-dC interact at low temperatures by the formation of intermolecular hydrogen bonded dimers. Noncomplementary molecules such as pdG-dT, pdT-dG, pdG-dG, pdA-dC, and pdC-dC do not self-associate by the formation of intermolecular hydrogen bonds under the present experimental conditions. The chemical shifts of the amino protons and the base protons are consistent with the interaction of two complementary dinucleotides to form a miniature double helix. An analysis of the chemical shift of the guanine amino proton resonance as a function of dinucleotide concentration has provided approximate dimerization constants. These results show that the stability of the miniature double helices is in the order (pdG-dG)·(pdC-dC) $\gtrsim$ (pdG-dC)·(pdG-dC) > (pdC-dG)·(pdC-dG) > (pdG-dT)·(pdA-dC) which reflects the effect of nucleotide sequence (and composition) on helix stability.

The study of the structure and function of nucleic acids has been an important area of research for the past 100 years. During the last few years a great deal of information has been obtained by a study of the properties of oligonucleotides by a variety of spectroscopic techniques. Nuclear magnetic resonance (NMR) and especially proton magnetic resonance (^{1}H NMR) have been especially useful in providing valuable information on the structure and interactions of mononucleotides, dinucleotides, and oligonucleotides (for a detailed review of the literature, see Ts'o (1974a,b) and Danyluk (1975)). The hydrogen-bonding properties of the nucleotide bases have been studied in *nonaqueous* environments (e.g., Hamlin et al., 1965; Katz and Penman, 1966; Kyogoku et al., 1966; Pitha et al., 1966; Shoup et al., 1966; Newmark and Cantor, 1968) and in the solid state (e.g., see the review by Sobell, 1969). In *aqueous* solutions the stacking interactions of the mononucleotides predominate and it is very difficult to observe base pairing. However, Raszka and Kaplan (1972) observed downfield shifts (<0.15 ppm) of the nucleotide amino resonances in concentrated mixtures of mononucleotides which is evidence for the formation of hydrogen-bonded complexes. We have recently exploited the use of deoxy and ribodinucleotides and dinucleoside monophosphates in the study of drug–nucleic acid complexes and have found that the dinucleotides seem to be good model compounds for DNA and RNA (e.g., Krugh, 1972; Krugh and Neely, 1973; Krugh et al., 1975; Krugh

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