the five model disulfides examined here χ_{SS} values of ~90° are favored. Consequently, S-S contributions to the negative CD band at 225-230 nm are likely to be minimal.

While NOE results permit assignment of type II β -turns to the Pro-D-Ala and Pro-Aib disulfides in CDCl₃, the CD spectra of all five disulfides are similar in both apolar (dioxane) and polar (MeOH) solvents. It thus appears that CD spectral types may not provide an unequivocal way of characterizing β -turn conformations.^{3,9} This point is further emphasized by the differences in sign of the $n \rightarrow \pi^*$ CD band in the acyclic and cyclic Pro-D-Ala peptides, both of which exhibit NOE's indicative of type II β -turns. The results of the present study also establish differences in the type of Pro-X β -turn conformation, preferred in the acyclic and cyclic peptides, for X = Gly and L-Leu. For X = Aib, both classes of peptides provide evidence for solvent-dependent equilibria between type II and type III structures. The Pro-D-Ala sequence

alone maintains the type II β -turn structure in the cyclic and acyclic peptides.

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Registry No. Piv-Pro-Pro-Aib-NHMe, 87587-51-7; Piv-Pro-Pro-D-Ala-NHMe, 87587-52-8; Piv-Pro-Pro-Gly-NHMe, 87587-53-9; Piv-Pro-Cro-Leu-NHMe, 87587-54-0; Piv-Pro-Pro-Val-NHMe, 87587-55-1; Boc-Cys-Pro-Gly-Cys-NHMe cyclic disulfide, 81007-01-4; Boc-Cys-Pro-Ala-Cys-NH-Me cyclic disulfide, 81006-99-7; Boc-Cys-Pro-D-Ala-Cys-NHMe cyclic disulfide, 81007-00-3; Boc-Cys-Pro-Aib-Cys-NHMe cyclic disulfide, 81006-98-6; Boc-Cys-Pro-Leu-Cys-NHMe cyclic disulfide, 87597-06-6.

Practical Synthesis of 5-Phospho-D-ribosyl α -1-Pyrophosphate (PRPP): Enzymatic Routes from Ribose 5-Phosphate or Ribose¹

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Abstract: This paper describes enzymatic syntheses of 5-phospho-D-ribosyl α -1-pyrophosphate (PRPP) on a 75-mmol scale. The reactions used PAN-immobilized PRPP synthetase as catalyst with in situ ATP-cofactor regeneration. In one procedure pure r-5-P was used as a starting material; in a second, r-5-P was synthesized by ribokinase-catalyzed phosphorylation of D-ribose and used in situ. The potential for use of PRPP as a starting material for the preparation of nucleotides was demonstrated by an enzymatic synthesis of UMP. This paper also describes several methods for the preparation of r-5-P: acid-catalyzed hydrolysis of AMP, acid-catalyzed hydrolysis of a crude mononucleotide mixture obtained by digestion of RNA, chemical synthesis from D-ribose, and ribokinase-catalyzed synthesis from D-ribose. Procedures are described for the isolation of PRPP synthetase (from Salmonella typhimurium) and ribokinase (from Lactobacillus plantarum) and for the immobilization of these enzymes in PAN.

5-Phospho-D-ribosyl α -1-pyrophosphate (PRPP) serves as a key intermediate in the biosynthesis of purine,⁵ pyrimidine,⁶ and pyridine⁷ nucleotides and of histidine⁸ and tryptophan.⁹ We are interested in synthetic routes to certain of these substances, especially the nucleotide cofactors (ATP, UTP, GTP, CTP, NAD-(P)(H)) required in enzyme-catalyzed organic synthesis.¹⁰⁻¹³ We were therefore interested in preparations of PRPP, which might be useful in practical-scale synthesis.

Although PRPP is commercially available, it is too expensive $(\sim$ \$76000/mol) to be used in practical syntheses. The high cost of PRPP is partially due to its intrinsic instabilility. At acidic pH

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Scheme I. Enzymatic Synthesis of PRPP from r-5-P



it decomposes in solution to ribose 5-phosphate (r-5-P) and inorganic pyrophosphate, while at alkaline pH, especially in the presence of divalent cations, the compound yields 5-phosphoribose cyclic 1,2-phosphate and inorganic phosphate.14 It is also

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thermally unstable. As a result of the sensitivity of PRPP, synthetic routes to it that could be coupled directly to its utilization in situ seemed particularly attractive.

PRPP has been synthetized chemically by Tener and Khorana,¹⁵ but this synthesis is not easily amenable to large-scale use. Biosynthesis of PRPP involves the reaction of r-5-P with ATP catalyzed by the enzyme ATP:D-ribose-5-phosphate pyrophosphotransferase (EC 2.7.6.1, PRPP synthetase).¹⁶⁻²¹ Uses of this enzyme for the preparation of PRPP have been common^{17,22,23} but have been conducted only on 0.1- to 1-mmol scales. This paper describes a larger scale synthesis of PRPP using immobilized PRPP synthetase and in situ ATP-cofactor regeneration (Scheme I). This method provides substantial amounts of PRPP (75 mmol) and opens new routes to nucleotides and their analogues.

Results and Discussion

Ribose 5-Phosphate. The r-5-P required in most of our syntheses of PRPP was prepared by acid-catalyzed hydrolysis of AMP.^{24a} This procedure affords r-5-P in 94% yield and 85-90% purity by a straightforward procedure. It is easily applicable to 100-g quantities of r-5-P. Because AMP is moderately expensive,^{24b} we have also explored three alternative synthetic routes to r-5-P using less expensive starting materials: acid-catalyzed hydrolysis of a crude nucleotide mixture obtained by nuclease P₁-catalyzed hydrolysis of RNA,²⁵ chemical synthesis from Dribose, and enzyme-catalyzed synthesis from D-ribose (Scheme II). In the first of these procedures, the crude mixture of AMP, GMP, UMP, CMP, other minor nucleotides, and residual oligonucleotides was subjected to Dowex 50 (H⁺) catalyzed hydrolysis. The acid-labile purine nucleotides (AMP and GMP) hydrolyzed, the resulting solution contained r-5-P and unhydrolyzed pyrimidine nucleotides. The nucleotides were removed by adsorption onto charcoal, and Na2r-5-P was isolated in 50%

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Scheme III. Synthesis of Ribulose-1,5-bisphosphate



yield based on the quantities of purine nucleotides present in the crude RNA digest. The pyrimidine nucleotides UMP (51%) and CMP (12%) could also be isolated by elution from the charcoal.

Ribose is also a useful starting material for synthesis of r-5-P,²⁶ and we have studied its chemical and enzymatic phosphorylation. Reaction of unprotected ribose with phosphorous oxychloride in the presence of 2,6-lutidine in triethyl phosphate gave only a 5% yield of r-5-P.²⁷ Phosphorylation of the readily available methyl 2,3-O-isopropylidene- β -D-ribofuranoside²⁸ (1, Scheme II) with POCl₃ and pyridine in acetonitrile, followed by hydrolysis of the intermediate 5-phosphorodichloridate, gave essentially quantitative conversion (>97%) to r-5-P. The product could be isolated as its monobarium salt in 69% yield, or as its disodium salt in 90% yield. The purity of the r-5-P obtained by these procedures is low $(\sim 60\%$ by weight in the barium salt, and $\sim 40\%$ by weight in the disodium salt) compared with that of the r-5-P obtained by acid-catalyzed hydrolysis of AMP (85-90% purity).29 The major impurities in these materials are, however, simply phosphate salts, and do not interfere in most enzyme-catalyzed reactions that use r-5-P as a starting material. It was, therefore, unnecessary to purify these materials further for our purposes.

Thus, for example, Na₂r-5-P obtained by chemical phosphorylation of ribose served as a satisfactory reactant in the synthesis of ribulose 1,5-bis(phosphate) (RuBP) outlined in Scheme III. This procedure differs from that reported previously³⁰ in two respects. First, ATP was regenerated by using the system based on pyruvate kinase and phosphoenol pyruvate¹⁰ rather than that using acetate kinase and acetyl phosphate. Second, the operating pH was set at 7.5 instead of 7.8 in order to minimize isomerization of the product into xylulose 1,5-bis(phosphate) (XuBP). The reaction was performed starting with 45 mmol of Na₂r-5-P (obtained from 50 mmol of ribofuranoside 1) and afforded 37 mmol (82% yield) of Ba_2RuBP in 42% purity. The product contained 0.07% of Ba₂XuBP by weight by enzymatic assay.³⁰

Enzymatic phosphorylation of unprotected ribose was accom-plished by using ribokinase³¹⁻³⁴ (RK, EC 2.7.1.17), ATP, and in

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R-5-F

Xu-5-P

"NY M^M

HUN,

Α

в

Ru-5-P



Pi

Figure 1. Proton-decoupled "P spectra of the product obtained from the RK-catalyzed synthesis of r-5-P (A), and the product after treatment with $Ba(OH)_2$. Samples were transformed from the monobarium salt to the disodium salt and dissolved in 50 mM Hepes-KOH, pH 7.0, containing 10 mM EDTA and 20% ${}^{2}H_2O$.

situ cofactor regeneration. We isolated RK from Lactobacillus plantarum, which had been adapted to growth on a medium containing D-ribose as the major carbon source.³¹ The enzyme was partially purified to a specific activity of 0.3 U/mg of protein and was immobilized in PAN³⁵ in 67% yield. Unfortunately, this enzyme preparation also contained as impurities phosphoriboisomerase (PRI, EC 5.3.1.6), which converts r-5-P to ribulose 5-phosphate (Ru-5-P), and ribulose 5-phosphate epimerase (Ru5PE, EC 5.1.3.1), which transforms Ru-5-P to xylulose 5phosphate (Xu-5-P). We did not attempt to separate these enzymatic activities, but used the crude mixture directly. Conversion starting with 100 mmol of D-ribose yielded, following isolating of the sugar phosphates as their salts, a mixture of 36 mmol of r-5-P, 15 mmol of Ru-5-P, and 38 mmol of Xu-5-P.³⁶ Ru-5-P and Xu-5-P are base labile and can be selectively destroyed in the presence of r-5-P.³⁷ Thus, treatment of the mixture with 0.15 N barium hydroxide afforded 31 mmol of r-5-P of 73% purity (Figure 1). The mixture of pentose phosphates is, however, usable directly for the synthesis of PRPP (see below) when this latter step is carried out in the presence of PRI and Ru5PE, since these enzymes convert the undesired sugars back to r-5-P as it is depleted by reaction.

Of the several preparations of r-5-P explored, that based on hydrolysis of AMP is clearly the most convenient, and the price of AMP is a consideration only for synthesis on the multimole scale. The other synthesis of r-5-P examined have both advantages and disadvantages relative to the acid-catalyzed hydrolysis of AMP. The preparation that starts from the RNA digest is basically similar to the preparation from AMP, but has the advantage that it uses a less expensive starting material. Moreover, it yields UMP as a potentially useful byproduct. Its disadvantages are that an additional enzymatic step is required to hydrolyze the

RNA to the mixture of nucleoside monophosphates and that the final isolation of r-5-P is more elaborate. The synthesis based on chemical phosphorylation of ribose employs inexpensive starting materials and straightforward chemical manipulations. Its disadvantages are the requirement for a chemical protection step and the fact that it generates r-5-P containing large quantities of phosphate. The direct enzymatic phosphorylation of D-ribose by RK is in principle the most attractive procedure. The enzyme has good stability and adequate specific activity and can be immobilized in good yield. The procedure we have used for its preparation is not, however, a particularly convenient one for several reasons. First, it requires initial adaptation of Lactobacillus to a ribose-based medium. Second, the enzyme yield is only modest (50-100 U from a 25-L fermentation). Third, the large quantities of ribose that are required for the fermentation would be expensive without the direct access we enjoyed to technical, fermentation-derived ribose. Fourth, the contaminating enzymatic activities in this preparation make it inconvenient as the catalyst for synthesis of pure r-5-P (although it serves adequately for a synthesis of PRPP). With an improved method of preparation of RK, this procedure would, however, probably be the method of choice.

PRPP Synthetase. PRPP synthetase was isolated from a strain of *Salmonella typhimurium* supplied to us by Switzer.³⁸ This enzyme has been purified to homogeneity and thoroughly studied by Switzer and co-workers.³⁹ The enzyme is aggregated: the molecular weight of a subunit is $\sim 31\,000$ and it exists mainly as pentamers. It requires Mg²⁺ and phosphate for activity, and its specific activity is 90 U/mg. The enzyme is quite stable when stored in 50 mM phosphate buffer and can be easily manipulated. We have used partially purified enzyme having a specific activity 25 U/mg. This enzyme was immobilized in PAN gel³⁵ in 80% yield.

Syntheses and Use of PRPP. The synthesis of PRPP from the pure r-5-P obtained from AMP was straightforward. In a typical synthesis, the reaction mixture contained r-5-P, PEP, magnesium chloride, inorganic phosphate, EDTA, a catalytic amount of ATP, and the enzymes PRPP synthetase, PK, and adenylate kinase (AK). All enzymes were separately immobilized in PAN.³⁵ The ATP required for the synthesis was regenerated in situ by the PEP/AK/PK system.¹⁰ The reaction was carried out on 100 mmol of r-5-P at 30 °C. Although the pH optimum for PRPP synthetase lies between 8.1 and 8.6,²¹ the synthesis of PRPP developed here was performed at pH 7.4. At this value of pH the enzyme has about 40% of its maximum activity but the stability of the PRPP formed is much enhanced. Using these reaction conditions a solution containing 75 mmol of PRPP was obtained after 4 days. The catalytic amount of ATP present in the solution was removed by adsorption on charcoal, and the solution containing the PRPP was either employed immediately in a subsequent transformation or kept frozen until use. In frozen solution at pH 7.0, PRPP shows ~6% decomposition/week at -20 °C.¹⁴ The PAN-immobilized enzymes were recovered from the reaction mixture with good retention of activity and were employed in subsequent preparations.

A second preparation of PRPP used the mixture of sugar phosphates obtained on RK-catalyzed phosphorylation of ribose in a coupled-enzyme system: that is, the synthesis of r-5-P (and its equilibration to Ru-5-P and Xu-5-P) were carried on in the same flask as its conversion to PRPP. The equilibrium constants for the reactions catalyzed by PRI and Ru5PE are close to unity³⁶ and thus the conversion of ribose to r-5-P, even with RK of the purity we used, can be coupled to the conversion of r-5-P to PRPP.⁴⁰ This procedure constitutes a "one-pot" conversion of ribose to PRPP (Scheme IV). The reaction mixture contained D-ribose, a catalytic amount of ATP, magnesium chloride, EDTA, DTT, PEP, inorganic phosphate, and the immobilized enzymes

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Scheme IV. Coupled Enzymatic Synthesis of PRPP from D-Ribose



PK, AK, RK, and PRPP synthetase. The reaction started with 100 mmol of D-ribose and afforded 74 mmol (assayed in solution) of PRPP. The product was precipitated as the dibarium salt and then transformed to the tetrasodium salt.⁴¹ The ³¹P NMR spectrum of the product obtained corresponded to that of authentic sample of PRPP (Figure 2). During this isolation, a significant part of the PRPP (~20%) was lost. For most purposes, however, isolation was not necessary and the crude mixture (after removal of ATP by charcoal) was a satisfactory source of PRPP for subsequent transformations.

As an illustrative synthesis utilizing PRPP, we have prepared uridine 5'-monophosphate (UMP). The formation in vivo of UMP from orotate takes place in two steps: condensation of orotate with PRPP and formation of orotidine 5'-monophosphate (O-5-P) (catalyzed by O-5-P-pyrophosphorylase, EC 2.4.2.10), and decarboxylation of O-5-P to UMP (catalyzed by O-5-P-decarboxylase, EC 4.1.1.23).⁶ These two enzymes are commercially available, but have low specific activity (O-5-P-pyrophosphorylase, ≈ 0.4 U/mg; O-5-P-decarboxylase, ≈ 0.3 U/mg). The synthesis was carried out by continuous slow addition of a PRPP solution to a suspension of orotate, magnesium chloride, DTT⁴² and PAN-immobilized O-5-P-pyrophosphorylase, O-5-P-decarboxylase, and inorganic pyrophosphatase (PP₁ase, EC 3.6.1.1.) (Scheme V). Using this procedure we obtained 22 mmol of UMP (73% yield) from a crude solution of 30 mmol of PRPP prepared from pure r-5-P.

Conclusion

This paper describes practical preparations of PRPP and demonstrates the use of PRPP in synthesis of a representative nucleoside monophosphate (UMP). The synthesis can be carried out starting from r-5-P, or by a one-pot procedure from D-ribose. Although PRPP synthetase is not commercially available, it is easily isolated in high specific activity, and the immobilized enzyme can be recovered from the reaction mixture and reused. The synthesis of PRPP starting from pure r-5-P is straightforward and probably the method of choice for most purposes. The second procedure for the preparation of PRPP from D-ribose is more



Figure 2. Proton-decoupled ³¹P NMR spectra of PRPP: (A) Authentic sample (Sigma); (B) isolated compound from the enzymatic synthesis from D-ribose. Samples were prepared in 50 mM Hepes-KOH, pH 8.0, containing 10 mM EDTA and 20% $^{2}H_{2}O$.

elaborate, since two enzymes (PRPP synthetase and RK) must be isolated. These isolation procedures are, however, simple, and partially purified enzymes are satisfactory for the synthesis.⁴³

The preparation of r-5-P by acid-catalyzed hydrolysis of AMP is the most convenient of the four methods explored. The other methods examined had, in principle, the advantage of using less expensive starting materials (RNA and D-ribose, rather than AMP). The preparation that starts from the RNA digest has also the advantage of yielding UMP as a useful byproduct. In this preparation, however, an additional enzymatic step is required, and the isolation procedure is more complicated. The chemical phosphorylation of D-ribose is straightforward, but this procedure suffers from its requirement for a protected ribose. The direct enzymatic phosphorylation of D-ribose catalyzed by RK would probably be the most attractive procedure for further development, if a better method of obtaining RK could be found.³³

The enzyme-catalyzed synthesis of UMP from the synthetic **PRPP** establishes that this material is suitable for use as a synthon in the preparation of nucleotides.

Experimental Section

General. Spectrophotometric measurements were performed at 25 °C on a Perkin-Elmer Model 552 spectrophotometer equipped with a thermostated cell. ³¹P NMR spectra were recorded at 121.5 MHz on a Bruker WM 300 spectrometer. Samples were prepared in 50 mM Hepes–KOH, pH 8.0, containing 10 mM EDTA and 20% ²H₂O (internal lock). Chemical shifts for ³¹P are reported with respect to external 85% H₃PO₄. HPLC was carried out on a Waters Associates system equipped with a differential ultraviolet detector operating at 254 nm, a differential refractometer detector, and a Resolve C₁₈ reversed-phase column (3.9 mm × 15 cm, 5-µm particle size, Waters). Elution was done with an aqueous solution of 0.5% dicyclohexylammonium phosphate, which was

⁽⁴¹⁾ Divalent cations are known to catalyze the decomposition of PRPP. Thus, the product is best stored as the tetrasodium salt. If desired, PRPP can be further purified by ion exchange chromatography; see ref 14.

⁽⁴²⁾ O-5-P decarboxylase is not fully active in the absence of thiol compounds.

⁽⁴³⁾ In the synthesis of PRPP directly from D-ribose, 3 equiv of PEP are needed. Recently developed large-scale syntheses of acetyl phosphate (disodium salt) would now probably make this the preferred system for ATP regeneration (Crans, D. C.; Whitesides, G. M. J. Org. Chem., in press. Kazlauskas, R.; Whitesides, G. M., unpublished data).

Scheme V. Synthesis of UMP from PRPP



adjusted to pH 7.0 with NaOH (flow rate 2.0 mL/min). A New Brunswick Model G-25 shaker-incubator and a New Brunswick Scientific Micro Ferm Model CMF-128S fermenter were used to grow cells. Cell growth was monitored by a Klett Summerson photometer. Cells were harvested by continuous centrifugation using a Sorvall KSB-R (Du Pont) continuous flow system. Cells were ruptured using a Aminco Rapid fill 20 000 psi French pressure cell. Protein concentrations were determined by the method of Warburg and Christian.⁴⁴

The enzymatic reactions were carried out in three-necked, roundbottomed flasks, which were modified with a port at the bottom to accommodate a pH electrode. Prior to the addition of the immobilized enzymes, solutions were deoxygenated with a stream of argon introduced via a gas dispersion tube. The enzymatic reactions were run at room temperature under argon unless otherwise specified. At the end of reactions the enzyme-containing gels were allowed to settle, and solutions were decanted under a positive argon pressure through a cannula. The gels were washed with 50 mM Hepes buffer, pH 7.4 and centrifuged, and the recovered enzyme activities were assayed.

Materials. Enzymes and biochemicals were obtained from Sigma. Dowex AG1-X8 and Dowex 50W-X8 were purchased from Bio-Rad. Phosphorous oxychloride, 2,2-dimethoxypropane and activated carbon (Darco, 100-325 mesh) were obtained from Aldrich. Na₂AMP (99% purity) was purchased from Kyowa Hakko USA, Inc. A 65% aqueous solution of technical D-ribose was a gift from Hoffmann-La-Roche. A stab of Salmonella typhimurium strain 422 was a generous gift from Dr. R. L. Switzer, University of Illinois, Urbana, and a freeze-dried sample of Lactobacillus plantarum ATCC 8041 strain 1242 (May 27, 1966) was obtained from The American Type Culture Collection. Phosphoenolpyruvate potassium salt (PEP⁻K⁺),¹⁰ PAN,³⁵ and methyl 2,3-isopropylidene- β -D-ribofuranose²⁸ were prepared according to published procedures.

Assay Methods.⁴⁵ Enzymes and biochemicals were assayed according to known procedures of Bergmeyer et al.⁴⁶ O-5-P-Pyrophosphorylase and O-5-P-decarboxylase were assayed according to the method of Umezu et al.⁴⁷

PRPP Synthetase. PRPP synthetase was assayed by a slight modification of the method of Ferrari.⁴⁸ The assay is based on the following coupled enzyme system:

$$ATP + r-5-P \xrightarrow{AKP} PRPP + AMP$$

$$AMP + ATP \xrightarrow{AK} 2ADP$$

$$2ADP + 2PEP \xrightarrow{PK} 2pyruvate + 2ATP$$

$$2pyruvate + 2NADH \xrightarrow{LDH} 2lactate + 2NAD^+$$

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mation of 1 µmol of product per min of 25 °C. (46) Bergmeyer, H. U. "Methods of Enzymatic Analysis"; Verlag Chemie: New York, 1974.

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$$U = \frac{OD}{\min \times 6.22} \times \frac{1}{2} \times \operatorname{assay vol} (mL)$$

PRPP. The determination of PRPP is based on the decrease in optical density at 295 nm upon the conversion of 5-fluoroorotate to 5-fluoro-UMP; 22

The reaction mixture (final volume 1 mL) contained Tris-HCl buffer, pH 8.5 (20 mM), MgCl₂ (2 mM), 5-fluoroorotic acid (0.2 mM), O-5-P-pyrophosphorylase (0.5 U), and O-5-P-decarboxylase (0.5 U). The initial absorbance was measured (E_1), and then the PRPP-containing sample (20 μ L) was added. The reaction was complete within 10 min and the final absorbance was measured (E_2). The total amount of PRPP was calculated from

$$PRPP \ (\mu mol) = \frac{E_1 - E_2}{3.8} DV$$

where D = dilution factor and V = volume of the original PRPP solution. **Ribokinase.** We have developed the following coupled-enzyme assay for RK:

ATP + D-ribose
$$\xrightarrow{RK}$$
 R-5-P + ADP
ADP + PEP \xrightarrow{PK} ATP + pyruvate
ruvate + NADH \xrightarrow{LDH} L-lactate + NAD

pу

The assay mixture (final volume 3 mL) contained triethanolamine buffer (70 mM, pH 7.3), PEP (0.4 mM), ATP (5 mM), MgSO₄ (8 mM), KCl (10 mM), NADH (0.2 mM), D-ribose (5 mM), LDH (10 U/mL), PK (7 U/mL), and an aliquot of RK (20-50 μ L). Units of activity were calculated according to

$$U = \frac{OD}{\min \times 6.22} \times assay \text{ vol } (mL)$$

An unknown reaction utilizing NADH was catalyzed by enzyme(s) present in the RK preparation. The assay was therefore first performed without ribose to obtain the rate of the reaction. This blank value was subtracted from the rate obtained when ribose was present.

R-5-P. The assay is based on a coupled-enzyme procedure which employs four enzymes.⁴⁹ The net reaction is as follows:

$2r-5-P + NAD^+ \rightarrow$ sedoheptulose-7-P+ 3-phosphoglycerate + NADH + H⁺

The amount of NADH produced is determined spectrophotometrically at 340 nm. When this method is used, 0.5 mol of NADH is obtained for each mol of r-5-P. The assay system, in a final volume of 1 mL, is composed of the following: glycylglycine buffer (25 mM), MgCl₂ (5 mM), thiamin pyrophosphate (0.01%), Na₂HAsO₄ (5 mM), NAD⁺ (5 mM), D-glyceraldehyde-3-P dehydrogenase (3 U), r-5-P isomerase (1 U), Xu-5-P epimerase (2 U), transketolase (0.5 U), and the r-5-P sample solution (20 μ L). Before transketolase is added, the initial absorbance is measured (E_1). After transketolase was added, the increase in absorbance was complete with 10–15 min (E_2). The amount of r-5-P is calculated by

r-5-P (
$$\mu$$
mols) = $\frac{E_2 - E_1}{6.22} 2DV$

where D = dilution factor and V = the volume of the original r-5-P solution. D-Glyceraldehyde-3-P dehydrogenase contains traces of LDH, which reoxidizes NADH in the presence of pyruvate. When the sample contains pyruvate, it is first treated with slight excess of NADH and LDH to convert the pyruvate to lactate. When the sample contains Ru-5-P and Xu-5-P in addition to r-5-P, they are determined in a separate assay⁴⁶ and the amount of r-5-P is obtained by subtraction from the value obtained in the above assay. Isolation of PRPP Synthetase.⁵⁰ Salmonella typhimurium Su 422

was cultured at 37 °C on a modification of the E Medium of Vogel and Bonner,⁵¹ which contained as follows (per liter): glucose, 30 g; Lmethionine, 0.6 g; K₂HPO₄, 10 g; MgSO₄·7H₂O, 2.0 g; citric acid, 4.0 g; Na₂(NH₄)PO₄, 10 g. Sterilized culture medium (20 mL) in a 250-mL Klett flask was innoculated with Salmonella typhimurium cells that had been previously grown on agar slants. The cells were grown at 37 °C with shaking to the middle exponential growth phase. The culture was then transferred to 1 L of culture medium, and cell growth was allowed to continue. When the middle exponential growth phase was reached again, the culture was used to inoculate 24 L of culture medium. The fermentation was carried out at 37 °C with aeration (8 L/min) and agitation (400 rpm). The pH was maintained at 7.0 by addition of a 28% solution of NH₄OH. Cells were harvested at the end of the exponential growth by continuous flow centrifugation. Approximately 800 g of wet cell paste was obtained. The cells were quickly frozen in liquid nitrogen and stored at -80 °C. Cells (200 g) were suspended in 800 mL of cold 50 mM K_2 HPO₄ buffer, pH 7.5, by agitation in a blender for 60 s. The suspension was passed twice through a continuous French press at 8000 psi. The extract was centrifuged at 23000g for 60 min. The supernatant (860 mL) contained 4031 units of PRPP synthetase activity. The subsequent steps (streptomycin sulfate-heat treatment, first ammonium sulfate fractionation, first acid precipitation, second ammonium sulfate fractionation, and second acid precipitation) were accomplished exactly as described by Switzer and Gibson.³⁹ This purification procedure afforded, after the second acid precipitation, 18 mL of a solution containing 890 units of PRPP synthetase activity (22% yield). The specific activity

of the enzyme was 25 U/mg. Isolation of Ribokinase.⁵⁰ Lactobacillus plantarum strain 124-2 (ATCC 8041) was grown and subcultured as previously described.⁵² Adaptation to D-ribose was performed on the following culture medium: 0.4% Difco yeast extract, 1% Difco nutrient broth, 1% sodium acetate, 0.01% MgSO₄, 0.001% NaCl, 0.001% FeSO₄, 0.001% MgSO₄, 1% Dribose, and 0.1% glucose. Cells were grown three times in the above culture and the last 1 L culture was used to inoculate a fermenter of 25 L. The fermenter was run at 37 °C without aeration and with the pH controlled at 7 with 10% NaOH. After 12 h the cells were harvested and washed with 0.9% NaCl. The yield was 48 g of wet cells.

Cells (24 g) were suspended in water (100 mL) and passed twice through a French pressure cell (10000 psi). The suspension (160 mL) obtained was centrifuged at 15000g for 30 min. The supernatant was separated, diluted with 100 mL of water, and treated with 20% streptomycin sulfate (4 mL). The mixture was stirred for 10 min and then centrifuged (15000g). To the supernatant 264 mL of $(NH_4)_2SO_4$ solution (92.7 g of $(NH_4)_2SO_4$, 55% saturation) was added over 30 min. and the solution was stirred for 40 min. The slight turbidity obtained was removed by centrifugation and the supernatant was treated with additional $(NH_4)_2SO_4$ (27.2 g, 70% saturation). The suspension was centrifuged (20000g) for 15 min, and the precipitate was collected and

dissolved in water (20 mL). The solution was dialyzed overnight against 3 L of 2 mM potassium phosphate buffer, pH 7.4. The dialized solution was diluted with 2 mM phosphate buffer (175 mL) and stirred with preequilibrated calcium phosphate gel (~360 mL). The gel was separated by centrifugation and eluted on a coarse sintered glass filter with 150 mL of 13 mM potassium phosphate buffer, pH 7.4, and then with 100 mL of the same buffer (20 mM). The enzyme was precipitated from both eluates with (NH₄)₂SO₄ (81 g and 148 g, respectively, 80% saturation); the combined precipitates were dissolved in 10 mM potassium phosphate buffer, pH 7.4, and dialyzed overnight against the same buffer (3 L). The enzyme solution was concentrated to 9 mL with an Amicon cell equipped with PM-10 membrane. The resulting solution contained 49 U of RK having specific activity of 0.3 U/mg. This enzyme solution was immediately subjected to immobilization.

Immobilization of PRPP Synthetase. PAN-1000 (6 g) was dissolved in 0.3 M Hepes buffer (24 mL, pH 7.6) containing ATP (3 mM), AMP (4 mM), r-5-P (5 mM), and PRPP (3 mM). The enzyme solution (5 mL) was added, followed by 0.5 M TET (5 mL). The resulting gel was ground and washed as described.³⁵ The immobilization yield was 80%.

Immobilization of Orotidine-5'-phosphate Pyrophosphorylase and Orotidine-5'-phosphate Decarboxylase. O-5-P-pyrophosphorylase and O-5-P-decarboxylase were immobilized separately. PAN-1000 (1 g) was dissolved in 0.3 M Hepes buffer (4 mL, pH 7.6) containing MgCl₂ (15 mM), PRPP (3 mM), orotic acid (3 mM), and UMP (3 mM). The enzyme solutions (1 mL) containing 5 mg of O-5-P-pyrophosphorylase and 4 mg of O-5-P-decarboxylase, respectively, were added followed by 0.5 M TET (0.72 mL). The resulting gel was worked up as described.³⁵ The immobilization yields for O-5-P-pyrophosphorylase and O-5-P-decarboxylase were 63% and 48%, respectively.

Immobilization of Ribokinase. PAN-1000 (10 g) was dissolved in 0.3 M Hepes buffer (22 mL) containing Na₂ATP (240 mg) and MgCl₂ (160 mg), with vigorous stirring. The ribokinase solution (9 mL, 49 U) was added, followed (after 30 s) by 0.5 M TET solution (7.5 mL). The mixture gelled within 1 min. It was kept at room temperature under argon for 30 min. The gel was transferred to a Waring blender with 40 mM Herpes buffer containing 40 mM (NH₄)₂SO₄ and was blended for 30 s. The gel was separated by centrifugation and washed twice with the same buffer without (NH₄)₂SO₄. The total activity of immobilized enzyme was 33 U (67% yield).

Preparations of r-5-P. Method 1: From AMP. Dowex 50 W-X8 (910 g, H⁺ form) was placed in a 4-L Erlenmeyer flask and distilled water was added to a total volume of 2.2 L. The flask was heated to boiling and stirred with an overhead stirrer. Na₂AMP (131 g. 262 mmol) was added to the flask rapidly (<2 min). The mixture was kept at boiling with stirring for 8.5 min and then immediately cooled in an ice-water bath. When the temperature of the mixture had dropped to 35 °C, the Dowex was removed by filtration. The filtrate was further cooled to 0 °C and the pH was ajusted to 7.5 with 10 N NaOH. The solution was concentrated on a rotary evaporator to ~1 L. Lyophilization of the concentrated solution yielded 79.9 g of pale yellow hygroscopic powder, which contained 246 mmol of Na₂r-5-P (94% yield) of 85% purity.

Method 2: From an RNA Digest. RNA (100 g, free acid, technical grade, from Torula yeast) was hydrolyzed enzymatically using soluble Nuclease P_1 as previously described.²⁵ The RNA digest contained (mmol) AMP (100), GMP (99), UMP (95), and CMP (73). This solution (2 L) was transferred to a 3-L three-necked flask equipped with a thermometer, a mechanical stirrer, and a condenser. Dowex 50 W-X8 (H⁺ form, 1 kg) was added, and the vigorously stirred mixture was heated to boiling. It was kept boiling for 7 min⁵³ and then immediately cooled in an ice-water bath. The resin was removed by filtration and washed with water. The pH of the dark brown solution was adjusted to 2.5-3.0, and the solution was stirred at 4 °C for 8 h with acid-washed charcoal (100 g). The charcoal was removed by filtration on Celite using a Büchner funnel and thoroughly washed with water. This procedure was repeated once more with fresh activated charcoal (100 g). The pH of the colorless solution obtained was adjusted to 7 and then it was lyophilized. The resulting colorless powder obtained (40.0 g) contained 105 mmol of r-5-P (50% yield based on the AMP + GMP present in the starting RNA digest). The material was 72% pure based on a molecular weight of 274 for anhydrous Na₂r-5-P.

The charcoal was washed with 3 L of 0.1 N NH₄OH in 50% aqueous ethanol. The brown solution obtained was neutralized and evaporated in vacuo. The solid obtained contained 48 mmol of UMP (HPLC retention time 4.2 min) and 9 mmol of CMP (HPLC retention time 3.3 min).⁵⁴ No AMP and GMP could be detected by HPLC. A sample of

⁽⁴⁹⁾ See ref 46, p 1342.

⁽⁵⁰⁾ All operations were performed at 0-4 °C unless otherwise mentioned (51) Vogel, H. J.; Bonner, D. M. J. Biol. Chem. **1956**, 218, 97-106.

⁽⁵²⁾ Stumpf, P. K.; Horecker, B. L. J. Biol. Chem. 1956, 218, 753-768.

⁽⁵³⁾ The formation of r-5-P was followed by HPLC (retention time 1.7 min).

⁽⁵⁴⁾ Most of the CMP stays on the Dowex, and it can be eluted with dilute HCl.

this solid (2 g) was further purified by chromatography on a Dowex AG1-X8 column (Cl⁻ form).⁵⁵ Elution of the nucleotides was performed with 2 mM HCl. Fractions containing UMP were collected, neutralized, and lyophilized to afford 813 mg of colorless solid, which contained 1.9 mmol of UMP (92% purity): ³¹P NMR [¹H], δ 3.7 and 2.3 (P_i); HPLC, retention time 4.2 min.

Method 3: Enzymatic Synthesis. To a 1-L solution containing Dribose (15 g, 100 mmol), Na₂ATP (3.0 g, 6 mmol), MgCl₂·6H₂O (3.0 g, 15 mmol), Na₂EDTA (330 mg, 1 mmol), DTT (150 mg, 1 mmol), and PEP-K⁺ (27.5 g, 120 mmol, 90% pure) was added PAN-immobilized RK (33 U) and PK (166 U). The pH of the solution was kept at 6.9-7.1 by occasional addition of a few drops of concentrated HCl. The course of the reaction was monitored by assays for r-5-P, Ru-5-P, and Xu-5-P. After 3.5 days the solution was separated from the gel. The residual activity of the enzymes (as % of the starting activities) were as follows: RK ~ 60 and PK 87. Acid-washed charcoal (40 g) was added to the solution, stirred for 3 h at 4 °C, and filtered over Celite. The solution obtained was treated with BaCl₂ (15 mmol) and filtered to remove inorganic phosphate. Addition of more $BaCl_2$ (100 mmol) followed by cold ethanol (1.5 volumes) afforded a colorless precipitate, which was collected by filtration, washed with cold ethanol, and dried in vacuo. This material contained 36 mmol of r-5-P, 15 mmol of Ru-5-P, and 38 mmol of Xu-5-P: ${}^{31}P$ NMR [${}^{1}H$] δ 4.61 (Xu-5-P), 4.59 (Ru-5-P), 4.10 (r-5-P), and 2.4 (P_i).

The r-5-P could be purified by selective basic hydrolysis of the alkali-labile pentuloses.³⁷ The mixture of barium salts prepared above was dissolved in 500 mL of 0.15 N Ba(OH)₂ and stirred at room temperature for 30 min. The mixture was neutralized with a solution of HBr and centrifuged to remove inorganic phosphate. To the cooled precipitate cold ethanol (1 volume) was added, and the resulting precipitate was filtered, washed with cold ethanol, and dried in vacuo. The colorless solid obtained (15.7 g) contained 31 mmol of r-5-P (73% pure): ³¹P NMR [¹H] δ 4.0 HPLC; retention time 1.7 min.

Method 4: Chemical Synthesis. Acetonitrile (125 mL) and freshly distilled POCl₃ (35 mL, 375 mmol) were added to a 1-L three-necked flask equipped with a thermometer and an addition funnel. The stirred solution was cooled in an ice-salt bath, and pyridine (20.3 mL, 250 mmol) was added over 15 min. Methyl 2,3-O-isopropylidene- β -D-ribofuranoside (1)²⁸ (51 g, 250 mmol) in acetonitrile (125 mL) was added dropwise to the cooled mixture at a rate such that the temperature did not exceed 0 °C. The addition took ca. 45 min, and the reaction mixture was stirred for an additional 2 h at 0 °C. An aliquot (25 mL) of the reaction mixture was hydrolyzed by addition of 1 mL of water and heating on a steam bath for 1 h; enzymatic assay indicated a 97% yield of r-5-P based on starting ribofuranoside. The reaction mixture was poured into 1 L of cold water and heated in a water bath at 70 °C for 1 h. It was then cooled in an ice bath, the pH was adjusted to 5.0 with 10 N NaOH, and 250 mL of a saturated aqueous solution of BaCl₂ was added. Precipitated solids (principally barium phosphate) were separated by centrifugation. The supernatant was cooled in an ice bath and its pH adjusted to 7.5. Ethanol (1.5 volumes) was slowly added to the stirred, cold solution. The precipitate was isolated by suction filtration, washed with ethanol, and dried to constant weight (103 g) in a desiccator. The resulting colorless solid contained 172 mmol of r-5-P (69%, based on starting ribofuranoside). The material was 61% pure by weight, based on a molecular weight of 365.5 for Bar-5-P.

The r-5-P was also isolated as its disodium salt. The hydrolyzed reaction mixture (prepared as described above) was cooled in an ice bath and its pH was adjusted to 7.4. The solution was concentrated to ca. 30% of its volume by rotary evaporation and then freeze-dried. The hygroscopic colorless solid obtained contained 225 mmol of Na₂r-5-P, corresponding to 90% yield based on starting ribofuranoside.

Enzymatic Synthesis of Ribulose-1,5-bisphosphate. A solution of Na₂r-5-P (45 mmol, prepared as described above from 50 mmol of 1), PEP⁻K⁺ (10.3 g, 45 mmol, 95% pure), MgCl₂·6H₂O (2 g, 10 mmol), DTT (460 mg, 3 mmol), and Na₂ATP (275 mg, 0.5 mmol) in 350 mL of doubly distilled water was adjusted to pH 7.15 and transferred to the reaction flask. A suspension of pyruvate kinase (PK, 276 U), phosphoribulokinase (PRuK, 156 U), and phosphoriboisomerase (PRI, 103 U), each separately immobilzed in PAN-1000 gel, was added. The reaction mixture was stirred under argon at ambient temperature and followed by enzymatic assay for r-5-P. After 18 h, no r-5-P remained. The enzyme activities recovered in the gels were as follows: PK, 93%; PRuK, 85%; PRI, 96%. The solution was cooled in an ice bath, and its pH adjusted to 4.5. A concentrated solution of BaCl₂ (22.9 g, 110 mmol in 85 mL of water) was added. The initial precipitate was separated by

centrifugation, and the pH of the solution ajusted to 6.5. Cold ethanol (an equal volume) was added slowly to this solution. The resulting precipitate was isolated by suction filtration and washed with ethanol. Drying in a desiccator over P_2O_5 afforded 51 g of colorless solid, which contained 36.8 mmol of Ba₂RuB (82% yield, 42% pure). It was contaminated with 0.006 mmol of (0.07%) Ba₂XuBP.³⁰

Synthesis of PRPP. Method 1: r-5-P as Starting Material. To a solution (1 L) containing Na₂r-5-P (33 g, 100 mmol, 83% purity), Na₂ATP (2.8 g, 5.6 mmol), MgCl₂·6H₂O (3.2 g, 16 mmol), PEP⁻K⁺ (46 g, 200 mmol, 90% pure), K₂HPO₄ (4.1 g, 30 mmol), and Na₂EDTA (336 mg, 1 mmol) were added separately immobilized PRPP synthetase (90 U), PK (180 U), and AK (250 U). The reaction was run at 30 °C, pH 7.4. After 4 days 75 mmol of PRPP had been formed. The solution was separated from the gel and stirred at 4 °C with activated charcoal (40 g). The charcoal was filtered over Celite and washed with cold water (400 mL). The resulting solution contained 70 mmol of PRPP and was stored at -80 °C. The recovered enzymes activities (as % of the starting activities) were as follows: PRPP synthetase, 82; PK, 72; AK, 60.

Method 2: D-Ribose as Starting Material. To a solution (700 mL) containing D-ribose (16 g. 100 mmol), Na2ATP (3.0 g, 6 mmol), Mg-Cl₂·6H₂O (3.0 g, 15 mmol), Na₂EDTA (336 mg, 1 mmol), DTT (154 mg, 1 mmol), PEP⁻K⁺ (68.7 g, 300 mmol, 90% pure), and KH₂PO₄ (6.8 g, 50 mmol) was added a suspension (370 mL) of PAN-immobilized PK (340 U), AK (210 U), RK (41 U), and PRPP synthetase (95 U). The reaction was conducted at pH 7.5 with occasional adjustment of pH with a few drops of concentrated HCl. The course of the reaction was followed by enzymatic assays for r-5-P, PRPP, and PEP. After 3 days, 74 mmol of PRPP had been generated. After separation from the gel, the solution was treated with activated charcoal $(2 \times 70 \text{ g})$. The resulting solution (1.35 L) contained 67 mmol of PRPP. To this solution was added BaCl₂ (30 mmol). The precipitate was removed by centrifugation. The supernatant was stirred with BaCl₂ (150 mmol) for 15 min at 0 °C and then subjected to centrifugation. The precipitate was added to a solution of Na₂SO₄ (180 mM, 1 L) and the mixture was stirred at 4 °C for 30 min. The precipitate of BaSO₄ was removed by filtration and the supernatant was lyophilized. The colorless solid obtained (47.8 g) contained 55 mmol of PRPP based on a molecular weight of 478.0 for anhydrous Na₄PRPP (55% pure). ³¹P NMR [¹H] δ 4.7 (5-P), 3.4 (P_i), -4.5 (1P_{β}, $J_{P_{\beta}-P_{\alpha}} = 22.0$ Hz), and -10.9 (1P_{α}, $J_{P_{\alpha}-P_{\beta}} = 22.0$ Hz).

Synthesis of UMP. To a suspension (0.5 L) containing sodium orotate (2.0 g, 11.2 mmol), MgCl₂·6H₂O (4.1 g, 20 mmol), and DTT (0.3 g, 2 mmol) were added PAN-immobilized O-5-P-decarboxylase (40 U), O-5-P-pyrophosphorylase (5 U), and PPiase (90 U). To this suspension, a solution of PRPP (0.5 L, 30 mmol maintained at 4 °C) was continuously added at a rate of 2-3 mL/h. The pH was maintained at 7.5 by addition of 2 N NaOH with a pH controller. Additional sodium orotate (3.4 g, 18.8 mmol) and MgCl₂·6H₂O (4.1 g, 20 mmol) were added after 4 days. After 8 days enzymatic assay and HPLC analysis indicated the presence of 25 mmol of UMP (83% yield, based on PRPP added). The gel was separated from the solution and the recovered enzyme activities were as follows: O-5-P-decarboxylase, 37%, and O-5-P-pyro-phosphorylase, 45%. The solution was stirred at 4 °C for 1 h in the presence of activated charcoal. The charcoal was filtered over Celite and washed with a solution of 0.1 N NH₄OH in 50% aqueous ethanol (4 L). The solution was neutralized and evaporated. The yellow solid obtained (28.6 g) contained 22 mmol of UMP. ³¹P NMR [¹H] δ 3.7 and 2.3 (P_i); HPLC, retention time 4.2 min.

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Note Added in Proof. The PRPP synthetase gene has been cloned [at The Institute of Biological Chemistry B at the University of Copenhagan] into *E. coli*, and the enzyme purified to near homogeneity in yields 20-40 times that obtained from *Salmonella typhimurium* Su 422: Hove-Jensen, B. *Abstr. Ann.*

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Registry No. 1, 4099-85-8; 1 (5-phosphorodichloridate), 87372-46-1; PRPP, 7540-64-9; PRPP synthetase, 9015-83-2; r-5-P, 4300-28-1; ribokinase, 9026-84-0; D-ribose, 50-69-1; UMP, 58-97-9; r-5-P-2Na, 18265-46-8; r-5-P-Ba, 15673-79-7; RuBP-2Ba, 82130-67-4; sodium orotate, 154-85-8; O-5-P, 2149-82-8; O-5-P-pyrophosphorylase, 9030-25-5; O-5-P-decarboxylase, 9024-62-8; PAN, 25014-41-9; AMP-2Na, 4578-31-8; PRPP-4Na, 87372-47-2.

Side Chain Conformations of Oxytocin and Vasopressin Studied by NMR Observation of Isotopic Isomers^{1a}

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Abstract: The side chain conformations of several residues of oxytocin and [8-arginine]vasopressin are compared by using measurements of the circumjacent vicinal couplings of ${}^{1}H^{\alpha}$, ${}^{13}C'$, and ${}^{15}N'$ to β protons and stereospecific β deuteration in series of 18 specifically designed and synthesized isotopic isomers. The conformation(s) of half-cystyls-1 and -6 and tyrosyl-2 is (are) markedly similar when comparing the two peptides, and other residues show only small differences. Conformational classes of side chain are identified. It is concluded that most of the side chain conformations are largely uninfluenced by the differences in the primary structures of the two peptides.

In attempting to understand more fully the time-dependent or dynamic conformations of peptides in solution, we have concentrated our attention on determining the distribution of rotamers about individual torsion angles in oxytocin and [8-arginine]vasopressin (AVP). In examining these angles and their rotamer

Cys ¹ - S Cys ⁶ - Pro ⁷	Tyr ² Ile ³ Gln ⁴ Asn ⁵	Oxytocin
Cys ¹ –	Tyr ² i Phe ³	[8-Arginine] Vasopressi
 S Cys ⁶ Pro ⁷	 Gln ⁴ Asn ⁵	
Arg -	Gly" – NH _o	

states, we have measured multiple homo- and heteronuclear coupling constants about the angles. This approach is well calibrated for staggered rotamers about the χ^1 angle in amino acids² and has been used to refine the solution conformation of valinomycin³ with the assumption that rigid torsion angles pertain for the backbone in that case. In addition, we have applied this approach to the χ^1 angles of the half-cystyl bridge in oxytocin.⁴

We present here data for the half-cystyl bridge in AVP and for several other side chains in both oxytocin and AVP.

In these peptides, the two $C^{\alpha}-C^{\beta}$ torsion angles, χ^{1} 's, of the cystine bridge are torsion angles in the 20-atom ring structure. Other χ^{1} 's determine the relative orientations of their respective side chains, and it had been generally assumed that rotations about these $C^{\alpha}-C^{\beta}$ bonds are relatively free.

Previous experimental and theoretical studies of the conformations of oxytocin and AVP in aqueous solution have suggested that an equilibrium exists between several conformers of the ring and of the side chains (reviewed in ref 4-6). Their precise conformational characterization presents a considerable challenge, because the NMR spectra are complex, because there are multiple possible solutions for derived geometries from measured NMR values, and because a completely general approach to derivation of dynamic structures of relatively flexible molecules is not yet at hand.⁷ In this paper we show that the multiple circumjacent coupling constants about the respective $C^{\alpha}-C^{\beta}$ bonds of several residues are concordant with averaging among staggered rotamers. In addition, we compare the derived apparent free energies of rotamers of several residues of oxytocin and AVP, appropriate model peptides, and free amino acids. Our conclusions are based on measurement of circumjacent vicinal couplings between protons on C^{β} and the C^{α} substituents ${}^{1}H^{\alpha}$, ${}^{13}C'$, and ${}^{15}N'$ and use of stereospecific deuteration of protons on C^{β} in 18 isotopic isomers of oxytocin and AVP that were synthesized by using standard techniques.

In conformational terms the results show that the C^{α} - C^{β} torsion angles of the half-cystyl residues of AVP are eclipsed and predominantly fixed, similar to the situation previously found in oxytocin.⁴ Probes in the C-terminal acyclic tripeptide indicate that noncovalent interactions with the cyclic portion are quite minimal. Differences in side chain conformation between oxytocin and AVP are few. At this level of detail, the conformations of

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