Chemistry of Natural Compounds, Vol. 41, No. 1, 2005

STRUCTURES OF THE PEPTIDE Leu-Pro-Tyr-Pro AND ITS DERIVATIVES AND THE NICOTINAMIDE PART OF NADPH BY A SEMI-EMPIRICAL PM3 METHOD

V. V. Pak,[1] M. Ku,[1] N. Li,[1] M. S. Kim,[1] T. D. Kasymova,[2] and D. Y. Kwon[1]

UDC 542.8:541

The capability of peptides Leu-Pro-Tyr-Pro (LPYP), Leu-Pro-Tyr-Pro-Arg (LPYPR), and Ser-Pro-Tyr-Pro-Arg (SPYPR) to occupy the part of the binding site ascribed to NADPH in the active center of 3-hydroxy-3-methylglutaryl-coenzyme-A-reductase was analyzed using results from a semi-empirical PM3 method. The similarity of the peptide structures to NADPH was determined by comparing the relative contribution from projections of selected bond lengths in the peptides in two mutually perpendicular planes to the corresponding bond lengths in the nicotinamide part of the substrate. The correlation coefficient between the calculated average values of the relative contributions of the bonds and the inhibitory activity of these peptides is rather high (R = 0.926). This indicates that these peptides can occupy the part of the binding site for NADPH in the active center of the enzyme.

Key words: Leu-Pro-Tyr-Pro peptide, hypocholesterinimic peptide, NADPH.

Isoprenoids comprise a large group of natural compounds that are formed in living cells from mevalonic acid and are involved in various cellular functions such as growth regulation of higher plants, fungi, and mammals, including sterol synthesis. Investigations of the mechanism of mevalonic acid synthesis using enzymes of *Saccharomyces cerevisiae* [1], *Pseudomonas mevalonii* [2], and hamster 3-hydroxy-3-methylglutaryl-coenzyme-A-reductase (HMGR) [3], which are key enzymes in sterol biosynthesis, established that the conversion of 3-hydroxy-3-metylglutaryl-coenzyme-A (HMGR-CoA) into mevalonate involves two molecules of nicotinamidedinucleotidephosphate (NADPH) and occurs through two sequential hydryl shifts. Although isoprenoids are necessary for normal cell functioning, an excess of certain products synthesized from mevalonate, e.g., cholesterol, can lead to progressive atherosclerosis and cardiovascular diseases associated with it.

Statins are known inhibitors that effectively lower the blood plasma level of cholesterol. They are widely prescribed for treating hypocholesterolemia [4]. All statins contain structures similar to HMG in their molecules [5-7]. Therefore, they may occupy the binding site for HMG and part of the binding surface for CoA. Thus, statins block access (HMGR-CoA) to the catalytic center of the enzyme. However, they do not occupy the binding site for NADPH.

Another class of compounds with hypocholesterolemic activity includes peptides. A peptide [Leu-Pro-Tyr-Pro-Arg (LPYPR)] and enterostatins [Val-Pro-Asp-Pro-Arg (VPDPR), Val-Pro-Gly-Pro-Arg (VPGPR), and Ala-Pro-Gly-Pro-Arg (APGPR)] are known as hypocholesterolemic peptides. The synthesis of three peptides and the study of their hypocholesterolemic activity, which was determined as the ability to inhibit HMGR, have been reported [8].

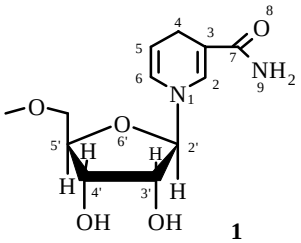
We examined the capability of LPYP, LPYPR, and SPYPR to occupy the binding site for NADPH. We started with the fact that these peptides contain a proline unit in the 2-position and four units from the N-terminus. Such a sequence may play an important role in HMGR inhibition. On the other hand, proline is the only unit with an aliphatic ring containing both the main and side chains. Its presence in the amino-acid sequence makes the conformation of the peptide relatively rigid. Thus,

1) Korea Food Research Institute, Songnam, Kyongki-do, 463-746, Korea, fax (8231) 709 98 76, e-mail: dykwon@kfri.re.kr; 2) S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (99871) 120 64 75. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 56-59, January-February, 2005. Original article submitted May 3, 2004.

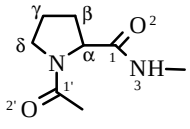
0009-3130/05/4101-0069

we assumed that proline in the above sequence can form a structure similar to the nicotinamide part of NADPH and, therefore, has similar interactions with the binding site for nicotinamide in HMGR.

We compared the peptide structures with those of the nicotinamide part of NADPH using data from an x-ray structure analysis of the enzyme active center with $NADP^+$ (**1**) [9] and selected four atoms (C4, O8, N9, O6′) with the shortest H-bond distances. The steric similarity of these structures were determined by calculating the percent of the projections of the selected bonds of the peptides to the corresponding bonds in the nicotinamide part of NADPH in two mutually perpendicular planes. The first plane passed through the pyridine ring of nicotinamide; the second, through C2′ of the ribose ring.



1



2

Structures of LPYP, LPYPR, and SPYPR and NADPH were calculated using the ChemOffice Desktop 2004 for Windows program set [CambridgeSoft (CS) Corporation] and CS MOPAC (version 1.11). The optimized structure parameters were obtained with a minimization gradient GRAD ≤ 0.01 kcal/mol. The calculated possible peptide conformations and corresponding heats of formation were determined by the rotation of torsion angle N-Cα-C1-N3 from 0 to 360° (**2**). Conformations of NADPH were found by rotating torsion angles C4′-C3′-C2′-N1 and C5′-C4′-C2′-N1 in the ranges 0-360° and 0-180°, respectively, and by changing the C5′-C2′-N1 bond angle from 90 to 180°.

The quantum-chemical method was selected by calculating possible LPYP conformations using the semi-empirical methods MNDO, AM1, and PM3. The results indicate that this peptide has five conformations according to MNDO and AM1 and nine conformations with local minima of heats of formation according to PM3 (Table 1). Structural features were determined by dividing them into four groups (A, B, C, F) describing the orientation of the peptide bond proposed in the previously described system [10]. The designations in this system are: "A" characterizes the conformation with torsion angles $-110° \le \varphi \le -40°$ and $-90° \le \psi \le -10°$; "B," $-180° \le \varphi \le -110°$ and $-40° \le \psi \le 20°$ or $-110° \le \varphi \le -40°$ and $-10° \le \psi \le 50°$; "C," $-110° \le \varphi \le -40°$ and $50° \le \psi \le 130°$; "F," $-110° \le \varphi \le -40°$ and $\psi \ge 130°$ or $\psi \le -140°$. Table 1 lists the calculated torsion angles (φ, ψ) and the relative heats of formation of the conformations according to the corresponding groups.

It can be seen that the peptide structures calculated using AM1 and PM3 belong to all four groups whereas conformations obtained using MNDO can be divided into groups A and F.

Experimental data for peptides containing proline are obtained mainly for the solid state [11-14]. Crystal structures of short peptides containing proline have the A conformations [15, 16]. Group C conformations, typically with a γ-turn, were observed in large polypeptide chains [17, 18]. F structures are found in polyprolines where several successive proline units facilitate formation of the peptide bond [11]. Including proline in the amino-acid sequence makes the peptide structure additionally rigid. Short proline-containing peptides have A conformations. Therefore, we concentrated on this group for further investigations.

A comparison of the heats of formation, torsion angles, and number of representative characteristic groups among the three methods showed that PM3 gives the most information regarding the peptide structures. Therefore, further structure calculations were made using this method.

Calculations of LPYPR and SPYPR structures using PM3 defined 12 local minima each for the heats of formation for each molecule. These were also divided into four characteristic conformation groups (Table 2).

In contrast with LPYP, the conformations with the lowest minima for these peptides were found in group A. Comparison of the torsion angles for the first two conformations of this group showed that they have high values for each peptide. Thus, we selected for the next investigation one structure from group A for each peptide. For LPYP, this was the first conformer of group A with torsion angles $\varphi = -98.43$ and $\psi = -20.24$; for LPYPR and SPYPR, conformations with the lowest minima in heats of formation and torsion angles $\varphi = -104.76$, $\psi = -44.91$ and $\varphi = -90.64$, $\psi = -61.09$, respectively.

TABLE 1. Optimized Torsion Angles φ and ψ and Relative Heats of Formation (kcal/mol) of Peptide LPYP Conformers Calculated by AM1, MNDO, and PM3 Methods

Group	Conformer		
	ΔH_f	C1′-N-C^{α}-C1	N-C^{α}-C1-N3
		AM1*	
C	0	-87.06	54.69
A	4.42	-91.62	-26.08
B	4.96	-83.64	2.53
F	8.41	-70.50	140.09
F	10.95	-64.48	162.88
		MNDO**	
F	0	-68.67	133.07
F	0.78	-66.79	135.12
F	1.70	-68.39	146.94
A	4.34	-73.01	-45.81
A	4.42	-76.40	-38.19
A	4.52	-77.17	-33.56
		PM3***	
C	0	-93.64	95.54
C	0.82	-62.76	108.41
C	1.77	-57.82	128.67
A	2.29	-98.43	-20.24
B	2.42	-106.58	13.00
A	2.65	-108.77	-78.50
F	3.84	-64.26	168.54
F	4.24	-61.06	149.14
F	6.04	-68.97	156.53

*The "0" value corresponds to ΔH_f = 238.17434 kcal/mol.

**The "0" value corresponds to ΔH_f = 222.16661 kcal/mol.

***The "0" value corresponds to ΔH_f = 256.84869 kcal/mol.

The NAD^+ and NADH structures were previously investigated for the gas phase using *ab initio* Hartree—Fock methods [19]. The reduced and oxidized forms of nicotinamide in vacuum and solution have also been compared using this same method [20, 21]. The calculations showed that the oxidized form of nicotinamide in solution will be found primarily in the *cis*-(a)-form whereas the reduced form has the *trans*-(b)-form. In this instance, the *cis*- and *trans*-forms refer to the orientation of the nicotinamide carboxyamide O relative to the pyridine N.

The transition energy from the *cis*- to the *trans*-form of NADPH was calculated as 12 kJ/mol. According to x-ray structure analysis, the preferred structure for the oxidized form of nicotinamide in the crystal is the *cis*-form. However, analysis of the investigated structures of NADPH-dependent enzymes [22] showed that $NADP^+$ cannot adopt its optimal conformation in the enzyme active center. This may be one reason why $NADP^+$ is replaced by another NADPH molecule. Thus, we structured the whole next investigation on the *trans*-form of the nicotinamide part of NADPH.

TABLE 2. Optimized Torsion Angles φ and ψ and Relative Heats of Formation (kcal/mol) for LPYPR and SPYPR Peptide Conformers Calculated by the PM3 Method

Group	Conformer		
	ΔH_f	C1′-N-C^{α}-C1	N-C^{α}-C1-N3
		LPYPR*	
A	0	-104.76	-44.91
C	0.32	-89.06	63.58
C	2.74	-82.36	113.49
C	4.52	-89.22	77.07
C	4.64	-56.43	129.17
A	9.37	-107.06	-45.01
A	9.87	-103.85	-49.17
A	11.33	-97.42	-40.91
A	13.48	-84.01	-49.06
F	15.24	-58.89	149.20
F	17.37	-60.33	156.92
F	17.40	-97.09	-140.86
		SPYPR**	
A	0	-90.64	-61.09
A	0.54	-94.61	-64.40
B	3.49	-65.02	0.90
C	6.97	-93.91	104.09
C	8.63	-86.08	68.62
C	10.50	-71.44	165.91
C	13.69	-87.05	70.01
A	14.89	-98.86	-48.27
C	16.00	-61.52	124.26
A	16.05	-104.42	-57.41
F	22.77	-67.79	174.60
C	28.94	-74.59	127.51

*The "0" value corresponds to ΔH_f = 162.62402 kcal/mol.
**The "0" value corresponds to ΔH_f = 184.47785 kcal/mol.

The calculations of NADPH defined 21 conformations with local minima of heats of formation, the first five of which are listed in Table 3.

Calculations of the *trans*-form of NADPH [22] showed that the amide bond lies practically in the same plane that passes through the pyridine ring of the nicotinamide. According to our calculations, the deviation of the C2-C3-C7-N9 torsion angle obtained for the conformation with the lowest minimum in the heat of formation is -0.2° from this plane. This agrees well with the results from the *ab initio* method. Since the O atom of the ribose ring is not coplanar with the nicotinamide, we compared its position relative to it using the structures of the two first conformers and determined that the difference in the torsion angles is only 5°, which indicates the structures are similar in this part of NADPH.

Projections of the peptide bond lengths in selected fragments were calculated to compare the peptide structures in the nicotinamide part of NADPH and to compare them with the corresponding projections of the bonds in the nicotinamide part of NADPH using two mutually perpendicular planes. The first three bonds C7-O8, C7-N9, and C3-C4 are situated in the same plane and were compared with the peptide C1-O2, C1-N3, and C^{α}-C^{β} bonds, respectively (**1** and **2**). The C2′-O6′ bond, which lies in another plane, is comparable with the corresponding peptide C1′-O2′ bond. Then, the percent of the corresponding bond projections relative to the nicotinamide part of NADPH was calculated using the projections of the peptide and NADPH bond lengths (Table 4).

TABLE 3. Optimized Torsion Angles φ and ψ and Relative Heats of Formation (kcal/mol) for NADPH Conformers Calculated by the PM3 Method

Conformer	1	2	3	4	5
ΔH_f	0	5.56	11.62	17.07	32.67
C4′-C3′-C2′-N1	126.48	120.29	143.59	148.63	147.81
C3′-C2′-N1-C2	100.32	95.23	93.90	158.62	96.75

The "0" value corresponds to ΔH_f = 850.27905 kcal/mol.

TABLE 4. Bond Lengths for Peptides LPYP, LPYPR, and SPYPR as Percents of the Corresponding NADPH Bond Lengths

Bond	LPYP	LPYPR	SPYPR
	%		
C7-O8	76.98	75.00	50.32
C7-N9	92.64	70.55	47.53
C3-C4	80.93	47.88	51.56
C2′-O6′	65.55	60.99	50.94

TABLE 5. Average Percent Bonds, *Correlation Coefficient (R), and Inhibitory Activity for Peptides LPYP, LPYPR, and SPYPR

Atom position	LPYP	LPYPR	SPYPR	R
	%			
O8,N9,C4,O6′	79.22	63.11	46.53	0.9260
O8,N9,C4	83.82	63.74	50.25	0.87.18
O8,N9,O6′	78.48	68.54	44.64	0.9839
O8,C4,O6′	74.54	60.52	46.19	0.9223
N9,C4,O6′	79.85	59.76	45.47	0.8832
O8,N9	85.44	72.59	49.52	0.9706
O8,C4	79.32	60.27	51.11	0.8171
O8,O6′	70.26	66.94	42.75	0.9993
N9,C4	86.79	59.11	49.63	0.7747
N9,O6′	79.12	65.78	42.22	0.9719
C4,O6′	73.52	54.42	44.45	0.8349
Inhibition	31.00	30.00	14.00	

The average percent bond is the weighted average of the bond lengths in selected positions of the peptides as percents of the corresponding bonds in the nicotinic part of NADPH.

It can be seen that the agreement is best for LPYP and worst for SPYPR. Then we calculated the correlation coefficients (R) between the resulting percent bonds using all possible combinations (2, 3, and 4) of peptide-bond projections and the inhibitory activity of these peptides (Table 5).

It was found that the correlation coefficients were largest for two O positions (O8, O6′); for three bonds, for O8, N9, and O6′. In general, comparing correlation coefficients showed that the most important and well correlated positions are the

O atoms and carboxyamide N group and the ribose-ring O of the nicotinamide part of NADPH. The correlation coefficient obtained for all four positions was rather high, indicating good agreement between the activity and the peptide structures.

Thus, the results indicate that these peptides have structural features similar to the nicotinamide part in the studied positions and correlate with the inhibitory ability of these peptides. Therefore, they may bind to the active center of HMGR and interact similarly with it as NADPH. Also, LPYP, LPYPR, and SPYPR may occupy the part of the binding site for NADPH in the active center of the enzyme.

ACKNOWLEDGMENT

The investigation was supported by a Project of Functional Products and Development (2003) of the Korean Institute for Science and Technology Evaluation and Planning (KISTEP) of the Ministry of Science and Technology of Korea.

REFERENCES

1. D. Veloso, W. W. Cleland, and J. W. Porter, *Biochemistry*, **20**, 887 (1981).
2. B. G. Darnay, Y. Wang, and V. W. Rodwell, *J. Biol. Chem.*, **267**, 15064 (1992).
3. K. Flimpong and V. W. Rodwell, *J. Biol. Chem.*, **269**, 1217 (1994a).
4. A. M. Gotto Jr., *Am. J. Cardiol.*, **79**, 1663 (1997).
5. E. S. Istvan and J. Deisenhofer, *Biochim. Biophys. Acta*, **1529**, 9 (2000).
6. E. I. Wilding, J. R. Brown, A. P. Bryant, A. F. Chalker, D. J. Holmes, K. A. Ingraham, S. Iordanescu, C. Y. So, A. Rosenberg, and M. N. Gwynn, *J. Bacteriol.*, **182**, 4319 (2000).
7. E. S. Istvan, *Atherosclerosis Suppl.*, **4**, 3 (2003).
8. D. Y. Kwon, S. W. Oh, J. S. Lee, H. J. Yang, S. H. Lee, J. H. Lee, Y. B. Lee, and H. S. Sohn, *Food Sci. Biotechnol.*, **11**, 55 (2002).
9. E. S. Istvan, M. Palnitkar, S. K. Buchanan, and J. Deisenhofer, *EMBO J.*, **19**, 819 (2000).
10. S. S. Zimmerman, M. S. Pottle, G. Nemethy, and H. A. Scheraga, *Macromolecules*, **10**, 1 (1977).
11. E. Benedetti, A. Bavoso, B. di Blasio, V. Pavone, and C. Pedone, *Biopolymers*, **22**, 305 (1983).
12. G. Nomethy, K. D. Gibson, K. A. Palmer, C. N. Yoon, G. Paterlini, A. Zagari, S. Rumsey, and H. A. Scheraga, *J. Phys. Chem.*, **96**, 6472 (1992).
13. D. F. De Tar and N. P. Luthra, *J. Am. Chem. Soc.*, **99**, 1232 (1977).
14. K. A. Thomasson and J. Applequist, *Biopolymers*, **30**, 437 (1990).
15. B. N. Rao, A. Kumar, H. Balaram, A. Ravi, and P. Balaram, *J. Am. Chem. Soc.*, **105**, 7423 (1983).
16. E. Benedetti, A. Christensen, C. Gilon, W. Fuller, and M. Goodman, *Biopolymers*, **15**, 2523 (1976).
17. V. Madison, M. Atreyi, C. M. Deber, and E. R. Blount, *J. Am. Chem. Soc.*, **96**, 6725 (1974).
18. I. L. Karle, *J. Am. Chem. Soc.*, **100**, 1286 (1978).
19. Y. D. Wu and K. N. Houk, *J. Org. Chem.*, **58**, 2043 (1993).
20. H. Li and B. M. Goldstein, *J. Med. Chem.*, **35**, 3560 (1992).
21. G. D. Luca, T. Marino, T. Mineva, N. Russo, and M. Toscano, *Theochem-J. Mol. Struct.*, **501-502**, 215 (2000).
22. V. I. Polshakov, R. R. Biekofsky, B. Birdsall, and J. Feeney, *J. Mol. Struct.*, **602-603**, 257 (2002).