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STRUCTURE—ACTIVITY RELATIONSHIPS OF THE PEPTIDE Ile–Ala–Val–Pro AND ITS DERIVATIVES REVEALED USING THE SEMI-EMPIRICAL AM1 METHOD

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Several peptides with hypocholesterinemic properties were investigated in order to reveal structure—activity relationships. The semi-empirical AM1 method and molecular dynamics were used to determine common structural properties of these peptides. A mathematical model of the structure—activity relationship was obtained. According to this model, a hydrophobic part of these peptides is a required structural element for their biological activity. The proline acts as a key component in these compounds.

Key words: Ile–Ala–Val–Pro, hypocholesterinemic peptide, structure—activity relationship.

Hypocholesterinemia is one of the primary risk factors for damage to coronary arteries that has been linked to the development of various cardiovascular diseases [1, 2]. Treatment of such diseases comprises decreasing the level of circulating cholesterol and its synthesis, which involves mevalonic acid.

The biosynthesis of mevalonate in tissues involves 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA), two molecules of nicotinamide-adenine-dinucleotide (NADH) or its close analog nicotinamide-adenine-dinucleotide phosphate (NADPH), and 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR). The activity of this enzyme is the rate-determining step in the biosynthesis of cholesterol. It is controlled specifically by cholesterol and other products synthesized with mevalonate involvement [3-5].

HMGR can be inactivated by dehydration [6-8], phosphorylation [9-12], or application of inhibitors [13-15]. The last are currently being extensively investigated [16-20]. Therefore, several peptides with hypocholesterinemic properties were isolated from soy-protein glycinin. The three peptides Leu–Pro–Tyr–Pro, Leu–Pro–Tyr–Pro–Arg (LPYPR), and Ser–Pro–Tyr–Pro–Arg were synthesized chemically based on the amino-acid sequences obtained. Their hypocholesterinemic activities were investigated [21]. Then, another sequence, namely Ile–Ala–Val–Pro–Gly–Glu–Val–Ala (IAVPGEVA), was identified during enzymatic decomposition of glycinin by pepsin [22].

We synthesized seven peptides [Ile–Ala–Val–Pro (IAVP), Leu–Ile–Ala–Val–Pro (LIAVP), Ile–Ala–Val–Pro–Gly–Glu–Val–Ala (IAVPGEVA), Leu–Ile–Ala–Val–Pro–Gly–Glu–Val–Ala (LIAVPGEVA), Ile–Ala–Val–Pro–Thr–Gly–Val–Ala (IAVPTGVA), Leu–Ile–Ala–Val–Pro–Thr–Gly–Val–Ala (LIAVPTGVA), and Leu–Pro–Tyr–Pro–Arg (LPYPR)] that retained the IAVP fragment in all peptides and also LPYPR for comparison with previous investigations and analyzed their conformations. An in vitro test for measuring HMGR inhibition showed that all synthesized peptides possessed hypocholesterinemic properties. According to the results, the IC_{50} for IAVP, IAVPTGVA, and IAVPGEVA were measured as 59.3, 93.3, and 123.5 μM, respectively. For LIAVPGEVA, LPYPR, LIAVP, and LIAVPTGVA at 200 μM, the percent HMGR inhibition was 62.3, 33.7, 17.5, and 12.4%, respectively.

In the present work, which is a continuation of previous investigations, we attempted to find a structure—activity relationship of the synthesized peptides using the bioactivities, conformational analysis, and quantum-chemical calculations of their structures.

The activity as a function of structure was investigated in stages.

The structures of the peptides were calculated in the initial step using the semi-empirical AM1 method. Then, model structures of these compounds were constructed based on conformational analysis. The next steps were the selection of

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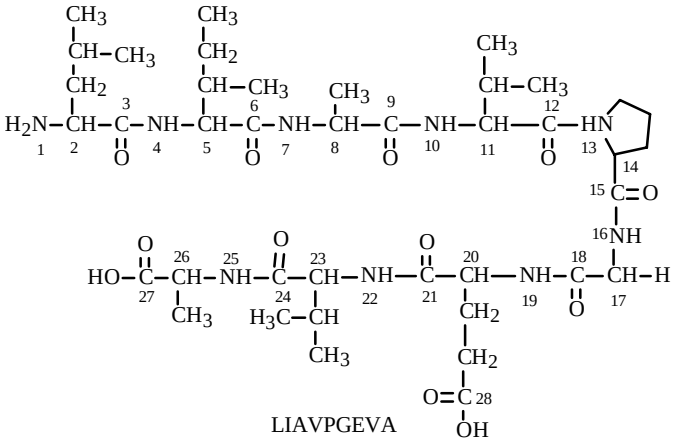
characteristic geometric parameters and their evaluation using molecular dynamics (MD). The final step was a quantitative evaluation of the relationship of the selected parameters on the activities of these peptides.

The peptide structures were calculated using the AM1 method [23] and the CS MOPAC program set (version 1.11) from the ChemOffice Desktop 2004 for Windows [CambridgeSoft (CS) Corporation, MA, USA]. We used a single atomic numbering of the peptide skeleton in order to compare the resulting structures.

All possible conformations were calculated by rotating the following torsion angles: for IAVP and LIAVP, torsion angle N7C8C9N10; for IAVPGEVA, LIAVPGEVA, IAVPTGVA, LIAVPTGVA, and LPYPR, N13C14C15N16 from 0 to 360°; and also for IAVPGEVA, LIAVPGEVA, and IAVPTGVA, LIAVPTGVA, C18N19C20C21 and C15N16C17C18.



According to the calculations, the number of observed local minima for IAVPGEVA, LIAVPGEVA, IAVPTGVA, LIAVPTGVA, IAVP, LIAVP, and LPYPR were 19, 21, 20, 22, 9, 11, and 5, respectively. The values of the angles ω obtained were close to 180°. This indicates that all peptides adopt the *trans*-peptide bond. A comparison of the torsion angles of the peptide skeleton in the conformation for the lowest minima of the heats of formation for IAVP and LIAVP showed that their three-dimensional structures were similar with the exception of both terminuses of these peptides. However, an analysis of the torsion angles for the IAVP peptide skeleton with the analogous angles for IAVPGEVA and IAVPTGVA showed that the GEVA fragment had a stronger effect on the IAVPGEVA structure than the TGVA part on IAVPTGVA. A similar effect was also observed for the conformations of LIAVP and LIAVPGEVA, LIAVPTGVA, respectively.

Model structures were constructed using data from the conformational analysis obtained by circular dichroism [24] and taking into account the behavior of the peptides on chromatography over a nonpolar stationary phase. According to the CD spectra [24], IAVP has a conformation with a first-order β-turn in the aqueous phase. It was also found that the GEVA and TGVA fragments made the main contribution to the disordered structure for IAVPGEVA, LIAVPGEVA and IAVPTGVA, LIAVPTGVA, respectively. The conformations in the IAVP- and LIAVP-fragments of these peptides did not change. An evaluation of the contribution from the leucine found that it was identical for LIAVP, LIAVPGEVA, and LIAVPTGVA compared with IVAP, IAVPGEVA, and IAVPTGVA.

Then, considering that the retention times for reversed-phase high-performance liquid chromatography (RP-HPLC) of the peptides correlate with their conformational structures [25-27] and that the hydrophobic phase can be thought of as a physicochemical model of biological systems that imitates the hydrophobic environment within protein [28], we hypothesized that the LIAVP and IAVP fragments made the main contribution to the hydrophobic interaction because the retention times for LIAVP, LIAVPGEVA, LIAVPTGVA, and IAVP, IAVPGEVA, IAVPTGVA were 10.76, 10.78, 10.85 and 9.10, 8.45, 8.59, respectively. The values were similar within these two groups of peptides.

Thus, model compounds were constructed based on the resulting conformation for IAVP in the lowest minimum of the heat of formation with all torsion angles of the peptide skeleton fixed between the Ile and Pro units for the other peptides containing this fragment and with the other geometric parameters optimized. For LPYPR, the energetically favorable conformation was selected because an analysis of this structure showed that it corresponded to conformations determined in crystal structures of short proline-containing peptides [29, 30].

In order to evaluate the steric similarities of the resulting model compounds, we selected sections between certain atoms and measured the distances between them. Table 1 lists the results.

TABLE 1. Spatial Similarities of Peptide Structures Compared Using Distances Between Selected C atoms in Model Compounds

Peptide	Distance, * Å						
	C2C15	C5C11	C5C15	C11C15	C11C20	C15C27	C15C28
IAVPGEVA		5.75	8.56	4.72	6.15	6.43	5.82
LIAVPGEVA	10.55	5.75	8.56	4.72	6.12	6.47	6.64
IAVPTGVA		5.75	8.56	4.72	6.89	4.65	3.57
LIAVPTGVA	10.49	5.75	8.56	4.72	6.46	5.28	3.44
IAVP		5.75	8.56	7.72			
LIAVP	10.53	5.75	8.56	4.72			
LPYPR				4.76	5.68		7.25

*Atomic numbering is given for the LIAVPGEVA structure.

TABLE 2. Selected Torsion Angle and Absolute Deviation* (α) from the Plane Passing Through the Proline Pyrrolidine Ring for Each Peptide

Peptide	Torsion angle and α, deg	
	N13C14C15O15	α
IAVPGEVA	-172.66	7.34
LIAVPGEVA	-169.56	10.44
IAVPTGVA	-131.45	48.55
LIAVPTGVA	-136.29	43.71
IAVP	168.15	11.85
LIAVP	167.27	12.73
LPYPR	124.97	55.03

*The α values were calculated as the difference between the selected torsion angle and an angle of 180°.

The distances C2C15, C5C11, C5C15, and C11C15 were selected as changes in the hydrophobic part of the peptide skeleton. Structural changes through side groups of glutamic acid, threonine, and tyrosine were estimated by C11C28 and C15C28; and the C-terminuses of these peptides, by measuring distances between C11 or C15 and C27. Table 1 shows that the distances between C11 and C20 in the VPGE-, VPTG- and LPYP-fragments are similar. This implies similar structures for the skeleton in this part of the peptides despite the different amino-acid sequences. Then, considering that the proline is placed in the fourth or fifth position from the N-terminus for IAVPGEVA, LIAVPGEVA, IAVPTGVA, LIAVPTGVA, IAVP, and LIAVP and in the second and fourth position for LPYPR, we hypothesized that it can play a definite role in the bioactivity of these compounds. However, IAVPGEVA, LIAVPGEVA, IAVPTGVA, and LIAVPTGVA have the β-turn conformation according to CD spectra. This often represents a bioactive structure in peptides and proteins. Therefore, we included measurements of the torsion angles (N13C14C15O15) of the proline carboxamide in the analysis of the biological activity of these compounds. Table 2 presents the torsion angles and their deviation (α) from the plane passing through the pyrrolidine ring of the proline.

We used MD to estimate structural changes of the resulting model compounds [31, 32]. The MD investigation was carried out using the same program set.

Table 3 presents the results from the MD investigation for the selected parameters.

TABLE 3. Selected Distances* Between C Atoms during the MD-Investigation

Peptide	Distance, Å					
	C2C15	C5C11	C11C15	C5C15	C15C27	C15C28
IAVPGEVA		5.8 (0.2)	4.3 (0.3)	8.2 (0.9)	7.6 (1.3)	6.7 (1.4)
LIAVPGEVA	8.6 (1.5)	5.0 (0.7)	4.0 (0.6)	7.8 (1.2)	5.5 (1.4)	5.3 (0.8)
IAVPTGVA		5.7 (0.2)	4.7 (0.1)	8.7 (0.5)	5.3 (0.9)	3.5 (0.3)
LIAVPTGVA	10.3 (0.5)	5.7 (0.3)	4.6 (0.2)	8.6 (0.2)	5.6 (0.8)	3.5 (0.2)
IAVP		5.8 (0.4)	4.9 (0.3)	8.5 (0.8)		
LIAVP	9.8 (1.3)	5.7 (0.2)	4.4 (0.3)	8.5 (0.5)		
LPYPR			4.4 (0.3)		6.0 (0.7)	6.8 (0.6)

*Average distance in Å and standard deviation in parentheses.
Atomic numbering for C is given for the LIAVPGEVA structure.

TABLE 4. Change of Characteristic Distances[1] Between α-C Atoms During the MD-Investigation

Peptide	Distance, Å					
	EED*	$d_{1.4}$**	$d_{2.5}$**	$d_{4.7}$**	$d_{5.8}$**	$d_{6.9}$**
IAVPGEVA	7.8 (1.3)		6.3 (0.6)	6.1 (0.4)	7.9 (0.5)	6.9 (1.6)
LIAVPGEVA	7.0 (1.4)	5.4 (1.4)	6.6 (1.0)	5.5 (0.8)	7.2 (1.3)	5.5 (1.1)
IAVPTGVA	11.0 (1.3)		6.2 (0.4)	5.9 (0.7)	6.9 (0.9)	6.2 (0.5)
LIAVPTGVA	11.2 (1.7)	6.3 (0.4)	6.5 (0.4)	6.1 (0.7)	6.6 (0.3)	6.2 (0.3)
IAVP	6.4 (0.8)		6.4 (0.8)			
LIAVP	8.7 (1.4)	6.8 (0.8)	6.6 (0.5)			
LPYPR	6.7 (1.0)			6.5 (1.0)		

[1]Average distance in Å and standard deviation in parentheses.
*EED is the distance between α-C atoms of the C- and N-terminuses of the peptides.
**$d_{i,i+3}$ is the distance between C_i^{α}- and C_{i+3}^{α}-atoms according to their numbering from the N-terminus.

The relative changes of the torsion angle (s) are given as the relative deviations compared with the change of the N13C14C15O15 torsion angle for LIAVPTGVA, which had the smallest change.

We investigated also the following interatomic distances in order to investigate conformational changes of the peptides under these conditions: 1) the distance between α-C atoms of the C- and N-terminuses of the peptides (EED), which approximately estimates its dimensions and level of packing and 2) the distance $d_{i,i+3}$ between two C^{α}-atoms in positions *i* and *i*+3, which indicate an ordered conformation if $d_{i,i+3}$ is less than 7 Å [33].

Table 4 shows that all peptides form a relatively stable turn between the fourth and seventh positions from the N-terminus. The average values for $d_{2,5}$ and $d_{6,9}$ indicate that a relatively ordered conformation is also observed in these fragments. However, more rigid structures were obtained for IAVPTGVA and LIAVPTGVA because their average distances and standard deviations are less than the corresponding values for the other peptides. LPYPR has a relatively more flexible structure.

According to MD results under these conditions, the resulting model structures do not convert to another ordered conformation and remain similar to the structure calculated by the AM1 method. Thus, the resulting model compounds and certain parameters were used for further analysis of the structure—activity relationship.

TABLE 5. Levels and Range of Change of Independent Parameters

Factor	Level and range of change	
	-1	1
x_1 (C2C15, C5C11, C11C15)	4.72-5.75	10.49-10.55
x_2 (C15C28)	0-3.57	5.82-7.33
x_3 (/180-∠N13C14C15O15/)	7.34-12.73	43.71-55.03

We used regression analysis with a 2^3 full-factor scheme [34] for a quantitative description of the structure—activity relationship. This enabled all independent factors to be varied and the effect of the interaction on changing the geometric structure parameters and their influence on the activity to be estimated. The selected independent factors (x_1, x_2, x_3) were: x_1, changes of the hydrophobic part (C2C15, C5C11, and C11C15); x_2, changes of the influence of the side groups of glutamic acid, threonine, and tyrosine through the C15C28 distance; and x_3, involvement of proline as the interaction of its carboxamide with the part of the peptide skeleton at the active center through changes of the N13C14C15O15 torsion angle. Based on the MD investigation, we made certain assumptions in determining both levels given in Table 5.

Using 2^3 full-matrix factor analysis, coefficients of the regression equation were calculated by a least-squares method. The resulting mathematical model for the structure—activity correlation has the form:

$$y = 30.16x_0 - 18.63x_1 - 8.06x_2 - 11.06x_3 + 12.11x_1x_2 + 2.63x_1x_3 - 2.73x_2x_3 - 4.41x_1x_2x_3.$$

Analysis of the mathematical model presupposes that the peptides interact with the active center of the enzyme and inhibit it through a "key—lock" model [35]. According to the calculations and MD investigations, all peptides adopt a β-turn conformation of various types in the amino-acid sequences –Pro–Gly–, –Pro–Thr–, and –Pro–Tyr–. This may occur because, as was investigated in several studies [36, 37], the –Pro–Gly– sequence is highly capable of forming a β-turn conformation, like the –Pro–Thr– sequence [38]. On the other hand, IAVPGEVA, LIAVPGEVA, IAVPTGVA, and LIAVPTGVA consist of two parts. One is relative rigid because it contains only hydrophobic amino acids (IAVP–, LIAVP–). The other is relatively flexible because it includes glutamic acid and threonine which probably also help to form this conformation. Changes of the peptide skeleton during the MD investigation indicate that the β-turn conformation and the small changes occurring in the hydrophobic fragments give rather large deviations in the flexible part of these model structures.

The aromatic radical in LPYPR probably increases the stability of the β-turn conformation due to a C–H - π-type interaction [39]. Thus, it can be assumed that the β-turn conformation plays a substantial role in the biological activities of these compounds.

Next, the mathematical model suggests a substantial effect from the hydrophobic part. This is seen in the significant difference in the bioactivities of IAVP and LIAVP. The reduced inhibiting capability of the peptides upon elongation of the amino-acid sequence by adding a leucine unit is probably due to a steric effect arising upon interaction with the HMGR binding site. A coefficient of secondary importance that reflects the effect of the proline carboxamide group indicates that this position plays a definite role in the activities of these peptides. Replacing the N atom in the carboxamide of –Pro–Gly–, –Pro–Thr–, and –Pro–Tyr– by an O atom for IAVP and LIAVP doesn't causes a loss of activity. The carboxyl of proline in IAVP and LIAVP, like the carboxamide for all other compounds, probably participates in the formation of H-bonds to the enzyme binding site. The spatial location of the side group also contributes to the bioactivity of the peptides. However, this effect is estimated to be smaller than the effect of hydrophobicity and the proline unit. Despite the fact that all peptides have structures similar to the –Pro–Gly–, –Pro–Thr–, and –Pro–Tyr– fragments, the ability to recognize them is different.

According to the MD investigation, the conformational sensitivities of these fragments are different and depend on the influence of the side group. Inclusion of the threonine unit for IAVPTGVA and LIAVPTGVA is reflected in the relatively limited flexibility in the –VPTG fragment compared with other peptides. However, they have higher EED values. Thus, the substantial difference in HMGR inhibition may be explained by a dependence on their location at the binding site. Regarding IAVPGEVA and LIAVPGEVA, the MD results showed that the –VPGE– fragments have relatively large conformational flexibilities. However, these peptides have more compact structures, which probably explains their similar hypocholesterinemic activities and, therefore, the relatively equal affinity for the enzyme active site.

According to the mathematical model, a change of hydrophobicity exerts an influence on the bioactivity of the peptides. The effect of proline on HMGR inhibition has almost the same effect as that of the double interaction of side groups of glutamic acid, threonine, and tyrosine with a change of hydrophobicity for each peptide. Then, interpolation of the mathematical model on the bioactivity increase suggests that the proline unit may involve a recognition-active element. The results suggest that a geometry close to the optimal one for forming H-bonds at the enzyme active site may be formed first through hydrophobic interaction with the binding site, which ensures the best orientation of the carboxyl, as observed for IAVP and, second, by adopting the β-turn conformation in the –VPGE–, –VPTG–, and –LPYP– fragments for the other peptides. However, in this instance the degree of similarity depends on the compactness of the peptide structure. The glutamic acid, threonine, and tyrosine side groups are proposed to participate in both formation of additional H-bonds that intensify the interaction with the binding site and the stabilization of the β-turn conformation.

Thus, we found certain characteristic features of the structures for the structure—activity relationships of the proline-containing model peptides by analyzing them, the MD investigation, and the mathematical model. Thus, the hydrophobic part is a required element for their bioactivity. The maximum length of this frgment is four amino acids. Furthermore, the proline unit is a key structural element and can be located at both the C-terminus and in any other position of the amino-acid sequence except the N-terminus. The formation of a conformation and a turn between proline in the i+n position and the next amino acid is also a necessary structural feature for activity. The placement in the i+n+1 or i+n+2 position of an amino acid with a negatively charged side group, depending on the length of the side chain, is also necessary for adopting a geometry cose to the optimal one for H-bonds to the binding site at the enzyme active center.

EXPERIMENTAL

Peptide structures were calculated using the AM1 method [23] and the CS MOPAC (version 1.11) program set from ChemOffice Desktop 2004 for Windows [CambridgeSoft (CS) Corporation, MA, USA]. Quantum-chemical structures were optimized by gradient minimization to 0.001 kcal/mol.

Structural changes of the resulting model compounds were evaluated using MD [31, 32]. The MD investigation was performed using the ChemOffice Desktop 2004 for Windows program set [CambridgeSoft (CS) Corporation, MA, USA]. The integration interval was 2 femtoseconds (1 femtosecond = 10^{-15} sec) at 300 K.

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