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Predictive bioinformatic identification of minor receptor group human rhinoviruses

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ABSTRACT

Major group HRVs bind intercellular adhesion molecule 1 and minor group HRVs bind members of the low-density lipoprotein receptor (LDLR) family for cell entry. Whereas the former share common sequence motives in their viral capsid proteins (VPs), in the latter only a lysine residue within the binding epitope in VP1 is conserved; this lysine is also present in "K-type" major group HRVs that fail to use LDLR for infection. By using the available sequences three-dimensional models of VP1 of all HRVs were built and binding energies, with respect to module 3 of the very-low-density lipoprotein receptor, were calculated. Based on the predicted affinities K-type HRVs and minor group HRVs were correctly classified.

1. Introduction

Human rhinoviruses, two established (A and B) and one proposed species (C) within the genus Enterovirus circulate as more than 100 types in the human population causing common colds. For the best characterized species A and B the receptors for host cell access are known; 12 types, the minor group, bind low-density lipoprotein receptor (LDLR), very-LDLR (VLDLR), and LDLR-related protein (LRP) and 87 types, the major group, bind intercellular adhesion molecule 1 (ICAM-1). All minor group HRVs are species A, whereas major group HRVs belong either to species A or B [1]. The recently discovered species C is poorly characterized biochemically and its receptor(s) is not known [2]. Recently, genome sequences of all known HRV serotypes and of several field isolates have been determined [3].

The genomic single stranded (+) RNA genome is enclosed within an icosahedral shell of 30 nm diameter composed of 60 copies of each of the capsid proteins VP1, VP2, VP3, and VP4. The binding sites of the respective receptors have been determined via electron cryo-microscopy and X-ray crystallography of complexes between virus and soluble receptor fragments. ICAM-1 binds within the canyon, a cleft encircling the fivefold axis of icosahedral symmetry [4] and contacts either of two motives conserved within each of the species (except from minor group HRVs that lack these motives) [5]. Conversely, as shown for the minor group virus HRV2, human VLDL-receptors attach via several of their ligand-binding repeats to the BC, DE, and HI loops of VP1 that build a star-shaped dome at the fivefold axis close to the icosahedral vertex [6]. Within the receptor footprint the 12 minor group HRVs only exhibit a common lysine residue at the tip of the HI-loop but the remaining residues are highly variable as they also contribute to the type-specific antigenic epitopes. Even when taking into account spatial vicinity within the three-dimensional structure, no obviously conserved amino acid pattern is apparent. An additional complication in understanding receptor recognition is the existence of 10 major group HRVs that also possess a lysine residue (and were therefore termed 'K-type HRVs') at a position equivalent to that of the lysine in minor group HRVs. Based on antigenic cross reactivity and sequence similarity HRV8 and HRV95 were combined into one single type [1]; however, since there are differences within the area equivalent to the receptor footprint they were considered separate types in the present communication. Like all other major group HRVs, K-types cannot infect via LDLR and/or LRP; they are neutralized by soluble ICAM-1 [7] and prevented from infecting HeLa cells by receptor

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blockage with a monoclonal antibody against ICAM-1 [8]. Phylogenetically, minor group viruses form three subclusters and K-types are combined in two subclusters and two outliers, HRV24 and HRV58; when compared to the similarity between other HRVs the analysis does not suggest a higher phylogenetic relationship between the clusters [3]. Only recently the basis of receptor specificity emerged as a combination of charge complementarity and hydrophobic interactions [9]. Reasoning that the 12 minor group HRVs are recognized by the same receptor, presumably via interactions that differ for each type, we attempted to distinguish the two rhinovirus groups (and in particular minor group and K-type HRVs) by using a simple, largely automatable and unbiased bioinformatic approach; the 3D-structures of the contact sites between receptor and VP1 were modelled for all rhinoviruses based on the protein sequences [1] and the theoretical binding energies were calculated by three different approaches. The best performing method correctly classified K-type and minor group HRVs with all the latter exhibiting higher calculated affinities towards the receptor. This demonstrates the utility of energy calculations for the identification of binding partners by using 3D homology models.

2. Methods

2.1. Modelling VP1

VP1 sequences of the 101 HRVs [1] were downloaded from the UniProt knowledgebase. Note that HRV87 is identical with the acid-sensitive enterovirus EV68 [10] and therefore it was not considered further. The sequences aligned with ClustalW were truncated by removal of 70 residues from the N-terminus and as many from the C-terminus as to leave 180 residues. The resulting sequences were submitted to SwissModel [11] in 'first approach mode' with default parameters by using a PERL script for automation. Except from HRV7 and HRV69, sound models were obtained for all HRVs. For the latter two, visual inspection revealed that the program could not correctly build the loops not even when the sequences were resubmitted to SwissModel in 'optimized project mode' [12] by using HRV14 as template. Therefore, they were excluded from further analysis. Finally, 3D models of all 98 VP1 proteins including the BC, DE, and HI loops making up the receptor-binding epitope were obtained.

2.2. Modelling VP1–VP1′–V3

Since the footprint of receptor module V3 extends over two symmetry-related copies of VP1, such VP1–VP1* 'dimers' were assembled by superposition onto the experimental structure of VP1–VP1* of HRV2 by the 'magic fit' routine in Swiss-Pdb Viewer (SDBV4.0; Ref. [13]) by using a script. The VP1-dimers were energy minimized (100 cycles, steepest descendent minimization method) and final structures of the respective VP1–VP1*–V3 complexes obtained by combination with the coordinates of V3 taken from the HRV2-V23 X-ray structure [6]. Coordinates of these complexes were again energy-minimized as above. Note that the Ca^{2+} was not considered because no force filed parameters were available in *SPDBV*. Modelling of the HRV70 and HRV91 receptor complexes did not result in reasonable structures as their BC loops clashed with V3; this problem was not solved by energy minimization. As they are typical major group HRVs and not K-type viruses, we made no further effort to improve the models and excluded them from further analyses.

2.3. Energy calculations

Models of VP1–VP1*–V3 were submitted to the *Dcomplex* [14] web server (http://sparks.informatics.iupui.edu/song/complex.html). Data were entered and results retrieved automatically by using a PERL script. Models were also submitted to the *FastContact2.0* web server (http://structure.pitt.edu/servers/fastcontact/) [15,16] manually entering and retrieving the data. A local copy of the *FastContact2.0* program, kindly provided by Carlos Camacho, was employed as well. This latter software does not include the *CHARMm19* minimizer, which is being used in the web based version.

3. Results and discussion

As known from the 3D structure of the complex between V23, a two-module fragment of human VLDLR, and HRV2, the receptor interacts with VP1 only. We thus limited our model building efforts to module V3 of the receptor and the latter viral capsid protein. To reduce calculation time, the first 70 residues of all aligned VP1 proteins were removed and only the next 180 residues were considered. The deleted amino acids do not take part in the interaction and are even not involved in extensive contacts with the symmetry related VP1*. Templates selected by *SwissModel* running in 'automatic mode' are listed in Table 1. In accordance with the phylogenetic relationships [5] the program automatically selected the PDB coordinates of the B-type viruses HRV3 and HRV14 as templates for modelling of VP1 of the other B-types. For modelling the A-types, coordinates of HRV1A, HRV2, or HRV16 were automatically chosen as templates. Regarding the receptor groups there was no particular preference of the minor group types for any of the type-A templates, whereas for the K-types only HRV1A was used. As expected, for those HRVs whose 3D coordinates were in the database (HRV1A, 2, 3, 14, and 16) the corresponding data were selected for model building. To assess the reliability of the approach, VP1 from the latter viruses with available structures were also modelled automatically by excluding their own coordinates as templates. As seen in Table 2, all the models were within less than 0.65 Å root mean square deviation (RMSD) for the backbone (and less than 0.74 Å with the side chains included) from the experimental structure indicating good quality of the models.

3.1. FastContact performs better than Dcomplex in calculation of the binding energies

Having verified that our approach resulted in 3D models very well matching the known X-ray structures, we assumed that the other models were plausible and close to reality. Thus, we next

Table 1
Templates automatically selected by SwissModel for modelling VP1 of all HRVs. Blue, minor group; red, genus A major group; green, K-types (all genus A); orange, genus B major group; grey, HRV70 and HRV91 whose modelling led to strong crashes with V3. Striped, HRVs whose 3D X-ray structures are available. Note that in case of HRV48 and HRV72 the structure of HRV14 containing the antiviral capsid-binding hydrophobic antiviral compound WIN 52084 was automatically selected for modelling (accession number 1rud).

HRV1A 1r1a				HRV 2 1fpn	HRV 3 1rhi	HRV14 1k5m	HRV16 1aym
1A	95	39	65	2	70	5	25
1B	98	41	66	23	91	6	62
29	11	43	68	30	3	14	10
31	12	46	71	49	4	27	16
44	13	50	73	9	17	37	21
47	15	51	74	32	26	48*	45
8	19	53	75	67	35	52*	77
18	20	55	76		42	72*	81
24	22	57	78		79	84	90
40	28	59	80		83	86	96
54	33	60	82		92	93	100
56	34	61	88		99	97	HANKS
58	36	63	89				
85	38	64	94			*1rud	