A conserved glutathione binding site in poliovirus is a target for antivirals and vaccine stabilisation

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Strategies to prevent the recurrence of poliovirus (PV) after eradication may utilise non-infectious, recombinant virus-like particle (VLP) vaccines. Despite clear advantages over inactivated or attenuated virus vaccines, instability of VLPs can compromise their immunogenicity. Glutathione (GSH), an important cellular reducing agent, is a crucial co-factor for the morphogenesis of enteroviruses, including PV. We report cryo-EM structures of GSH bound to PV serotype 3 VLPs showing that it can enhance particle stability. GSH binds the positively charged pocket at the interprotomer interface shown recently to bind GSH in enterovirus F3 and putative antiviral benzene sulphonamide compounds in other enteroviruses. We show, using high-resolution cryo-EM, the binding of a benzene sulphonamide compound with a PV serotype 2 VLP, consistent with antiviral activity through over-stabilizing the interprotomer pocket, preventing the capsid rearrangements necessary for viral infection. Collectively, these results suggest GSH or an analogous tight-binding antiviral offers the potential for stabilizing VLP vaccines.
The Enterovirus (EV) genus of the Picornaviridae family contains notable viral pathogens that cause various animal and human diseases and are regarded as potential zoonotic threats. These small non-enveloped RNA viruses contain 60 copies each of the viral proteins VP1-4 arranged to form an icosahedral protein capsid. VP1-3 each comprise a β-barrel with extended surface loops and C-termini that form the outer capsid surface. The N-termini of VP1-3, as well as the whole of VP4 (N-terminal to VP2 in the VP0 precursor), form a network on the inner surface. Several vaccines now exist against enteroviruses, notably against poliovirus (PV) serotypes 1–3 (PV1-3, of species EV-C) and EVA71,4,3; however, there are no licensed anti-enterovirus drugs. The most promising drug candidates to date have been compounds targeting a hydrophobic pocket internal to the β-barrel of capsid protein VP1.6,9 These ‘capsid binders’ or ‘pocket factors’ mimic a fatty acid moiety found naturally within this pocket, which maintains the native D-antigenic conformation. This natural lipid is normally dislodged on receptor engagement, leading to a structural shift involving radial expansion and the appearance of pores in the capsid, forming the ‘C-antigenic conformation’. By binding more tightly than the natural fatty acid, the action of pocket factors is to block virus-receptor interaction and uncoating. Such antivirals against enteroviruses were discovered many years ago and although one of these, pleconaril, entered clinical trials, it was not taken forward due to possible side effects, and since it was able to inhibit the in vitro replication of PV2 and PV3 but not that of PV1.19

The World Health Organization polio eradication campaign using inactivated (IPV) and attenuated oral (OPV) polio vaccines has massively reduced the number of poliomyelitis cases, taking the world close to eradication. However, post-eradication, new HPV vaccines will be required that are not derived from an infectious virus, such as virus-like particle (VLP) vaccines utilising the capacity for recombinantly expressed capsid proteins to self-assemble into empty viral capsids.11,12 Whilst indistinguishable in many respects, these empty capsids are inherently less stable than an infectious virus and prone to switching conformation from the native D-antigenic state to the less immunogenic C-antigenic structure.12 Specifically, these VLPs lack the stabilising interactions from encapsidated RNA and from the maturation cleavage of VP0, which results in the repositioning of the VP2 N and VP4 C-termini to form an internal scaffold. Stable capsid (SC) mutants have been developed for the VLPs of all three serotypes of PV (PV1 MahSC6b, PV2 MEFSC6b, PV3 SktSC8)14, and PV3 capsids with 8 mutations are as immunogenic as IPV in the absence of adjuvant. Furthermore, PV VLPs have been successfully produced in recombinant expression systems, establishing a proof-of-principle for their use as potential vaccine candidates for a post-polio eradication era.15–18 Whilst live virus vaccines remain in use, antivirals may also be required after polio eradication to control outbreaks of circulating vaccine-derived polioviruses.19,20

Glutathione is an abundant thiol peptide present in animal cells and plays a critical role in maintaining cellular redox potential.21,22 An imbalance in the ratio between reduced (GSH) and oxidised (GSSG) forms of glutathione is implicated in various enterovirus infections23 and depletion of cellular GSH levels using the GSH biosynthesis inhibitor L-buthionine sulfoximine (BSO)24,25 and the small molecule inhibitor TP219,26 blocks the assembly and morphogenesis of many enterovirus capsids. Mutations conferring GSH-independence map to the protomer interface between proteins VP1 and VP3 and are consistent with GSH binding being essential for the formation of pentameric subunits critical to assembly.24,27 A benzene sulphonamide compound (CP17) and derivatives thereof have been identified, which inhibit viral replication with micromolar affinity in a cell-based screening assay for a range of EVs, including PV1 and some rhinoviruses. For PV1, the derivative CP48 was more active than CP17. CP17 has been observed bound at a VP1–VP3 interprotomer cavity of Coxsackievirus B3 (CVB3) using cryo-electron microscopy (cryo-EM)28,29, the same cavity where GSH has been observed to bind the bovine enterovirus EVF3.30 This cavity is conserved in many but not all EVs and not generally across other picornaviruses. Furthermore, inhibition of GSH synthesis with BSO in EVF3-infected cells shows GSH dependency for virus growth and dose-dependent stabilisation by GSH of EVF3 virions has been demonstrated by thermal stability assays.31 This is consistent with earlier studies showing that GSH stabilises certain enteroviruses, including PV during viral morphogenesis, as well as being a potent stabiliser of OPV vaccine formulations from heat inactivation.31 More recently, CP48 has been shown bound to CVB4. Both CP17 and CP48 can act synergistically with pocket factors suggesting they have different antiviral mechanisms that can be exploited to develop anti-enteroviral therapies with improved efficacy and reduced side effects.28,29

Here we have used PV VLPs as a clinically relevant model system to better understand the GSH-binding site as a druggable pocket for the development of antivirals against EVs and as a site for the potential stabilisation of synthetic vaccine candidates. Using single-particle cryo-EM, we show GSH bound to the VP1–VP3 interprotomer pocket for the stabilised PV3-SC8 VLP. Furthermore, we show that GSH can stabilise VLPs of all serotypes of PV in the native D-antigenic conformation required for eliciting an immune response. In addition, a high-resolution cryo-EM analysis (at better than 2.0 Å resolution) is presented for CP17 bound to a VLP of wild-type (wt) PV2. It was, however, not possible to observe binding of CP48 using similar protocols. The interactions between CP17 and the ligand binding site are analysed and compared to those seen for CP17 and CP48 in CVB3 and CVB4, respectively, allowing key conserved residues to be identified. Sequence differences in the VP1–VP3 interprotomer cavity between PV and other EVs may explain altered binding orientations and specificities of the benzene sulphonamide derivatives observed in the wt PV2–CP17 complex studied here, compared to other EVs such as CVB3 and CVB4. Collectively, these results further increase understanding of the biological role of the VP1–VP3 interprotomer pocket, as well as that of a new potential antiviral drug class against EVs. Exploiting these findings opens up strategies for GSH or synthetic analogues thereof to be used for targeting the druggable pocket to stabilise PV VLPs, which may be important for next-generation vaccines.

Results
GSH binds the VP1–VP3 interprotomer pocket on the surface of the PV3-SC8 VLP. To investigate the structural basis of whether and how GSH binds to PV capsids, we chose to examine the complex of GSH with a previously characterised PV VLP, PV3-SC8. Since PV3-SC8 has previously been shown to bind pocket factors such as GPP3 in the VP1 hydrophobic pocket, we investigated in situ if GSH binding interferes with this stabi-

lisation mechanism by incubating a molar excess of GPP3 (VLP:compound molar ratio of 1:300) followed by GSH at 10 mM to form a ternary complex (PV3-SC8GPP3+GSH) with the PV3-SC8 VLP expressed in yeast.16 This complex was applied to EM grids that were rapidly vitrified for single-particle cryo-EM data collection. Image processing of a final set of 5364 particles yielded a 2.5 Å icosahedral reconstruction as assessed using the FSC 0.143 threshold criterion (Supplementary Fig. 1 and Tables 1, 2). The cryo-EM electron potential map revealed the VP0, VP1 and VP3 capsid protein subunits of the PV3-SC8 VLP to be well ordered.
and there was an unambiguous feature for bound GSH in the interprotomer pocket formed between VP1 subunits from two adjacent protomers and VP3 from a single protomer (Fig. 1a–e); the same surface pocket shown to bind GSH in bovine enterovirus EVF330. A structure of the unbound native antigenic form of the same yeast-expressed VLP (PDB ID 8ANW33) facilitated comparisons. The overall structure of the GSH-bound PV3-SC8 VLP (Fig. 1a, b, d) is essentially identical to the unbound native antigenic form of PV3-SC8 VLP expressed previously in the plant, mammalian and yeast cells15,18,33, with root-mean-square deviation (RMSD) in Cα atoms of 0.68, 1.39 and 0.77 Å, respectively (Supplementary Fig. 2a). As expected GPP3 was

| Table 1 Cryo-EM data collection and image processing. |
|--------------------------------------------------------|
| PV3-SC8GPP3 : GSH (EMD-15725) (PDB 8AYX)          | PV3-SC8pleconaril : GSH (EMD-15726) (PDB 8AYY) | wt PV2-CP17 (EMD-15727) (PDB 8AYZ) |
| **Data Collection**                                    |                                                      |                                   |
| Voltage (kV)                                          | 300                                                   | 300                               | 300                           |
| Magnification (×)                                     | 129629                                                | 129629                           | 60314                         |
| Defocus range (µm)                                    | −2.9 to −0.8                                          | −2.9 to −0.8                      | −2.3 to −0.8                   |
| Dose rate (e−/pixel/s)                                | 0.59                                                  | 35.79                             | 14.02                          |
| Frames                                               | 60                                                    | 25                                | 50                             |
| Frame length (s)                                      | 1.163                                                 | 0.046                             | 0.034                          |
| Total electron dose (e−/Å2)                          | 34.89                                                 | 35.29                             | 34.68                          |
| Micrographs                                          | 2503                                                  | 4706                              | 4750                           |
| **Data processing**                                   |                                                      |                                   |                                |
| Pixel size (Å)                                        | 1.08                                                  | 1.08                              | 0.829                          |
| Initial particles (no.)                              | 19622                                                 | 34523                             | 157002                         |
| Final particles (no.)                                 | 5364                                                  | 15275                             | 51518                          |
| Box size (pixels)                                     | 450                                                   | 450                               | 450                            |
| Symmetry                                             | I                                                      | I                                 | I                              |
| Accuracy of rotations (°)                            | 0.1325                                                | 0.1845                            | 0.1085                         |
| Accuracy of translations (Å)                         | 0.2160                                                | 0.3175                            | 0.1658                         |
| Resolution (Å)                                       | 2.54                                                  | 2.64                              | 1.88                           |
| Map sharpening B-factor (Å2)                          | −52.3                                                 | −72.2                             | −15.0                          |

| Table 2 Structure refinement and validation for the capsid protein (VPO, VP1 and VP3). |
|----------------------------------------------------------------------------------------|
| PV3-SC8GPP3 : GSH (EMD-15725) (PDB 8AYX)                                              | PV3-SC8pleconaril : GSH (EMD-15726) (PDB 8AYY) | wt PV2-CP17 (EMD-15727) (PDB 8AYZ) |
| **Model composition**                                                                  |                                                      |                                   |
| Non-hydrogen atoms                                                                    | 5863                                                  | 5857                             | 6565                           |
| Protein residues                                                                      | 736                                                    | 736                               | 802                            |
| Ligands                                                                               | GPP3:1                                                | Pleconaril:1                     | Sphingosine:1                  |
|                                                                                       | GSH:1                                                 | GSH:1                            | CP17:1                         |
| Waters                                                                                |                                                        |                                   |                                |
| **Refinement**                                                                        |                                                      |                                   |                                |
| Resolution (Å)                                                                         | 2.54                                                  | 2.64                              | 1.88                           |
| Map CCa (Mask)                                                                        | 0.85                                                  | 0.87                              | 0.90                           |
| Map CCa (Volume)                                                                      | 0.83                                                  | 0.85                              | 0.88                           |
| **RMS deviations**                                                                    |                                                      |                                   |                                |
| Bond lengths (Å)                                                                       | 0.002                                                 | 0.003                             | 0.003                          |
| Bond angles (°)                                                                        | 0.470                                                 | 0.455                             | 0.549                          |
| **Mean B-factor (Å2)**                                                                |                                                      |                                   |                                |
| Protein                                                                               | 20.36                                                 | 23.74                             | 24.16                          |
| Ligand                                                                                | 19.37                                                 | 23.69                             | 24.60                          |
| Water                                                                                 |                                                        | 23.26                             |                                |
| **Validation**                                                                        |                                                      |                                   |                                |
| Molprobity® score (percentile)                                                        | 1.02 (100th)                                          | 0.91 (100th)                     | 1.11 (100th)                   |
| Clashscore®, all atoms (percentile)                                                   | 2.42 (99th)                                           | 1.65 (99th)                      | 2.42 (99th)                    |
| Ramachandran favoured (%)                                                             | 98.20                                                 | 98.20                             | 97.60                          |
| Ramachandran allowed (%)                                                              | 1.80                                                  | 1.80                              | 2.40                           |
| Ramachandran outliers (%)                                                             | 0.00                                                  | 0.00                              | 0.00                           |
| Rotamer favoured (outliers) (%)                                                       | 98.29 (0.16)                                          | 98.13 (0.31)                     | 98.69 (0.15)                   |
| Cβ deviations >0.25 Å (%)                                                             | 0.00                                                  | 0.00                              | 0.00                           |
| CaBLAM outliers (%)                                                                   | 1.12                                                  | 1.26                              | 1.28                           |
| CA Geometry outliers (%)                                                              | 0.56                                                  | 0.28                              | 0.26                           |
| EMRinger® score                                                                       | 4.99                                                  | 4.90                              | 7.84                           |

aMap CC is given for the full particle reconstruction.

bWilliams et al. (2018) Protein Sci 27:293–31551.

cBarad et al. (2015) Nature Methods 12:943–94652.
observed bound within the hydrophobic pocket of VP1, in an essentially identical conformation to that previously observed for this compound bound to plant expressed PV3-SC8 (Fig. 1d, f and Supplementary Fig. 2b, c). This confirmed that the binding of GSH did not hinder the ability of the VP1 hydrophobic pocket to accommodate capsid-binding drugs such as GPP3, so effects at the two druggable sites, separated by some 22 Å in the capsid are likely to be additive. In the apo structure of PV3-SC8 (PDB ID 8ANW), no features were observed for GSH at the interprotomer pocket (Fig. 1c), or for GPP3 in the VP1 hydrophobic pocket, with the latter instead occupied by the naturally acquired lipid from the yeast cell, modelled as sphingosine based on the length of the carbon chain fitting the cryo-EM potential map. The lack of GSH in the apo structure most likely reflects a relatively fast off-rate, leading to its loss during extensive purification. We also confirmed these results in a parallel experiment by determining a 2.6 Å cryo-EM reconstruction of PV3-SC8 bound to GSH and an alternative VP1 hydrophobic pocket binding drug, pleconaril (Supplementary Figs. 1, 2d–g). The PV3-SC8GPP3+GSH and PV3-SC8pleconaril+GSH complexes are essentially identical in backbone conformation for the VP1, VP0 and VP3 subunits of the capsid protomer and binding mode of GSH, with a Cα RMSD of 0.19 Å between GPP3 + GSH and pleconaril + GSH forms of the complex (Supplementary Fig. 2a).

The GSH molecule observed bound in the VP1–VP3 surface pocket of the PV3-SC8 VLP is anchored in place by hydrogen bonds, salt-bridges and hydrophobic interactions (Fig. 2a, b). The carboxyl group of the GSH glycine moiety forms hydrogen bonds with two critical arginine residues; Arg257 and Arg242, contributed from neighbouring capsid protomers (Figs. 1d, 2a, b). Additional hydrogen bonds are formed between Asp169 and Gln174 of VP1 with the amide and carbonyl oxygen groups of the cysteine moiety of GSH, respectively (Fig. 2a, b).
GSH stabilises the C-terminus of VP3 to form the binding pocket. In apo PV3-SC8 (Fig. 1c) and other native PVs, the final three residues of the VP3 C-terminus (Leu236, Pro237 and Gln238) are disordered. In the PV3-SC8 GPP3 + GSH and PV3-SC8 pleconaril + GSH complexes, we observed that GSH binding resulted in some or all of these residues becoming ordered in the cryo-EM potential map, with Leu236 forming part of the binding site (Fig. 2c, d), so that hydrophobic interactions from Ser234, Ala235 and Leu236 of VP3 form a cap stabilising GSH in the interprotomer surface pocket (Fig. 2b, c). For PV3-SC8 GPP3 + GSH complex showing the final three residues are ordered upon GSH binding compared to the apo structure of PV3-SC8 without GSH bound (PDB ID 8ANW, EMD-15543). The electron potential map for VP3 is shown at 1.4 Å and rendered at 2 Å around atoms. VP1 subunits of protomer A and B are shown as molecular surfaces and coloured as in a and GPP3 bound in the VP1 pocket is shown as an orange stick model.

**Fig. 2** Glutathione interactions in the VP1-VP3 interprotomer pocket of the PV3-SC8 VLP and stabilisation of the VP3 C-terminus. a Cartoon representation of GSH (magenta stick model) bound in the interprotomer surface pocket between neighbouring capsid protomers of the PV3-SC8 VLP. VP1 and VP3 of protomer A are coloured light blue and light red, respectively, and VP1 of protomer B is coloured grey. Amino acid side chains for residues of VP1 and VP3 forming the GSH-binding pocket are shown as sticks and labelled. Hydrogen bond and salt-bridge interactions are shown as green dashes and distances labelled. b Ligplot+ representation of the GSH-binding pocket showing details of key interactions. Hydrogen bonds and salt-bridges are shown as green dashed lines and hydrophobic interactions as red arcs. c Cryo-EM electron potential map of the VP3 C-terminus (red) in the PV3-SC8 GPP3 + GSH complex showing the final three residues are ordered upon GSH binding compared to d the apo structure of PV3-SC8 without GSH bound (PDB ID 8ANW, EMD-15543). The electron potential map for VP3 is shown at 1.4 Å and rendered at 2 Å around atoms. VP1 subunits of protomer A and B are shown as molecular surfaces and coloured as in a and GPP3 bound in the VP1 pocket is shown as an orange stick model.

The GSH binding site is highly conserved in enteroviruses. The key interacting residues in the GSH-binding site are conserved across a panel of representative enteroviruses, including the three serotypes of PV and EVF (Fig. 3a, b). Notably, in VP1, Tyr259, Arg242, Arg257, Trp173 and Gln174 are strictly conserved and Asp169 is highly conserved (Fig. 3a). These residues form the core of the binding site and stabilise the bound GSH in the interprotomer pocket; with Arg242 and Arg257 of VP1 from neighbouring protomers in the interface conferring strong positive charge characteristics to the GSH-binding pocket and forming hydrogen bond and salt-bridge contacts with the carboxy terminus of GSH (Fig. 2b). This positively charged patch is a conserved feature in other EVs as seen in the EVF GSH complex (Fig. 3c, d). The Leu236 residue that forms hydrophobic interactions from the stabilised VP3 C-terminus with bound GSH in the pocket is chemically conserved across EVs along with other VP3 C-terminal residues (Fig. 3b). Figure 3c shows a structure-based phylogenetic tree of representative enteroviruses coloured according to the similarity of the GSH binding site.

GSH stabilises the C-terminus of VP3 to form the binding pocket. In apo PV3-SC8 (Fig. 1c) and other native PVs, the final three residues of the VP3 C-terminus (Leu236, Pro237 and Gln238) are disordered. In the PV3-SC8 GPP3 + GSH and PV3-SC8 pleconaril + GSH complexes, we observed that GSH binding resulted in some or all of these residues becoming ordered in the cryo-EM potential map, with Leu236 forming part of the binding site (Fig. 2c, d), so that hydrophobic interactions from Ser234, Ala235 and Leu236 of VP3 form a cap stabilising GSH in the interprotomer surface pocket (Fig. 2b, c). For PV3-SC8 GPP3 + GSH clear structure was observed in the cryo-EM map up to the end of the C-terminus (Gln238), whereas for PV3-SC8 pleconaril + GSH only an additional two residues (Leu236 and Pro237) became ordered upon GSH binding. For the apo form of PV3-SC8 with no GSH bound, no features were observed in the cryo-EM map beyond Ala235 (Fig. 2d). In total, 389 Å² of solvent-accessible surface area for GSH is buried in the interprotomer surface pocket, representing ~78 % of the total solvent-accessible surface area of the molecule.
contacting residues. The viruses labelled on this figure are reported to be dependent on, or stabilised by GSH, and cover all the major branches of the phylogenetic tree, however, there is a suggestion that the situation in some viruses, for example, EVA71, might be more complex.

GSH has a stabilising effect on PV VLPs in vitro. The role of GSH in facilitating virus assembly for enteroviruses like Coxsackieviruses and PV is well established, and the structural basis for this is now understood. To investigate the effect on the physico-chemical properties of VLPs rather than virus particles, the antigenic state of PV VLPs was investigated following heating in the presence of various concentrations of GSH (Fig. 4 and Table 3). Both yeast and mammalian (hamster, BHK-21) cells expressed PV VLPs were examined for selected examples of the three serotypes with and without stabilising mutations. GSH markedly increased the stability of the mammalian-expressed serotype 3 and serotype 2 VLPs (Fig. 4 and Table 3) and the serotype 1 and 2 yeast VLPs so that they became considerably more stable than IPV (Table 3), although surprisingly, there was little effect on the stability of the yeast-expressed PV3-SC8 particles (Table 3). This was unlikely to be because particles from yeast were already associated with GSH as the cryo-EM structure of purified yeast-derived PV3-SC8 particles showed that there was no specific chemical entity bound at the GSH-binding site (Fig. 1c). It may possibly reflect differences in the occupancy of the hydrophobic pocket in VP1, or at another site affecting stability, since the ternary complexes of PV3-SC8GPP3+GSH and PV3-SC8pleconaril+GSH demonstrate that the bound factors could

Fig. 3 Conservation of GSH-binding pocket in enteroviruses. a Multiple sequence alignment of the VP1 and b VP3 subunit sequences of PV3-SC8 with several major enteroviruses. Amino acid residues that form the GSH-binding site are marked with circles above the sequence and coloured blue (VP1 protomer A), grey (VP1 protomer B) and red (VP3 protomer A). Enterovirus species A, B, C, D, F and rhinovirus A and B are marked alongside the alignments. c, d Electrostatic charges (±5 kT e\(^{-1}\)) mapped onto the molecular surface of c PV3-SC8GPP3+GSH and d the EVF3 GSH complex and coloured from blue (positive charge) to red (negative charge). e Phylogenetic tree derived from a structure-based alignment of the representative set of major enteroviruses aligned in a. Those reported to be dependent/stabilised by GSH are marked: T where this was reported in reference, D where reported in reference 30 and B where reported in this paper. Abbreviations for each branch of the tree (PDB ID): CVA16 coxsackievirus A16 (5C4W), EVA71 enterovirus A71 (3VBH), CVB3 coxsackievirus B3 (6ZCL), CVB4 coxsackievirus B4 (6ZCK), CVA9 coxsackievirus A9 (1D4M), ECV1 echovirus 1 (1EV1), ECV7 echovirus 7 (2 × 51), ECV11 echovirus 11 (1H8T), PV1 poliovirus type 1 Mahoney (1HXS), PV2 poliovirus type 2 Lansing (1EAH), PV3 poliovirus type 3 Sabin (1PV), PV3-SC8GPP3+GSH poliovirus type 3 Saukett in complex with GPP3 and GSH (8AYX), EVD68 enterovirus D68 (4WM8), EVF3 enterovirus F3 (6T4C), HRVA16 human rhinovirus 16 (1AYM), HRVB14 human rhinovirus 16 (4RHV).
act additively or synergistically. The properties of the yeast-expressed PV3-SC8 particles were exceptional as GSH was found to stabilise all other particles tested irrespective of serotype, expression platform or whether stabilising mutations had been introduced (Table 3). Nearly all the stabilisation effect was observed at a GSH concentration of 1 mM, suggesting that this might be a practicable additive for vaccine formulation.

Other enterovirus inhibitors bind PV VLPs at the same interprotomer pocket as GSH. Recent work has shown a class of benzene sulphonamide compounds bound to enteroviruses28,29 at the same interprotomer surface pocket as GSH30. We investigated whether these compounds might also bind PV VLPs. Since GSH was shown to effectively stabilise wt PV2 VLPs (Table 3) they were soaked with a molar excess of the benzene sulphonamide derivative CP17 (VLP:CP17 molar ratio of 1:2500), applied to grids and vitrified for single-particle cryo-EM. After image processing, a final set of 51,518 particles yielded a 1.97 Å icosahedral reconstruction for the wt PV2-CP17 complex (FSC 0.143, Supplementary Fig. 3a, b). Ewald sphere correction35, yielded only a small improvement to 1.88 Å (see Methods, Table 1 and Supplementary Fig. 3a). The resultant electron potential maps revealed highly detailed features for the backbone and sidechain conformations of the VP0, VP1 and VP3 subunits of the capsid protein (Fig. 5a, b and Supplementary Fig. 3c). Initial maps sharpened with the automatically estimated B-factor of −53.6 Å² revealed a distinctive ‘L-shaped’ feature bound at the same interprotomer pocket occupied by GSH in the ternary complexes of PV3-SC8GPP3+GSH and PV3-SC8pleconaril+GSH, as well as the EVF3 GSH complex30. Maps sharpened less aggressively (B-factor of −15.0 Å²) confirmed unambiguously that this feature was due to the presence of CP17 bound at the interprotomer pocket of the wt PV2 VLP (Fig. 5a–c).

Like the binding mode for GSH, CP17 is bound within the VP1–VP3 interprotomer pocket of the wt PV2 VLP through two critical arginine residues—Arg243 and Arg258—from neighbouring VP1 subunits, which form hydrogen bonds and salt-bridges with the carbohydrate group of CP17 (Fig. 5d, e). The bulk of CP17 forms hydrophobic interactions with the network of residues forming the VP1–VP3 interprotomer pocket of the wt PV2 VLP (Fig. 5e). The wt PV2-CP17 structure is similar to that of the CVB3-CP17 complex (RMSD in Ca atoms of 1.1 Å)28,29, but there are differences in the precise orientation of CP17 as bound to wt PV2 VLP and CVB3 virus (Fig. 5f), although the key charge interactions with the carbohydrate group of CP17 are maintained in both cases. In the CVB3-CP17 structure, CP17 sits upright in the interprotomer pocket due to stacking interactions against Phe76 of VP1 and Phe236 of VP3 from CVB328. In the wt PV2-CP17 complex, the compound lies flat due to sequence differences between PV2 and CVB3, where VP1-Phe76 in CVB3 is replaced by VP1-Ile89 in wt PV2 (Fig. 5f). The interaction of the C-terminal residue VP3-Phe236 in CVB3 is absent in the wt PV2-CP17 structure as the C-terminus of VP3 is disordered in the latter, the final observed residue in wt PV2 being VP3 Ala235 (Fig. 5f). This enables CP17 to adopt a different conformation. The presence of Ala88 in VP1 of wt PV2 compared with Tyr75 at this position in the CVB3-CP17 structure avoids a steric clash that would otherwise disrupt the orientation of the compound in the wt PV2-CP17 complex (Fig. 5f). These sequence differences result in a change of ~52 ° in orientation between CP17 bound to CVB3 and wt PV2 (Fig. 5f). In total 520 Å² of solvent-accessible surface area for CP17 is buried in the interprotomer surface pocket of the wt PV2 VLP, representing ~87% of the total solvent-accessible surface area of the molecule.

Overall, GSH and CP17 occupy similar space within the VP1–VP3 interprotomer pockets of PV3-SC8 and wt PV2 VLPs, respectively, with substantial overlap between the volumes of the two compounds. In addition, the key points of interaction are broadly conserved, notably, the salt–bridge interactions between carboxylate groups on the ligands and arginine residues from adjacent VP1 subunits, stabilising the pentameric association of the protomers, as expected from the role of GSH in the assembly of the pentamers24, whilst elsewhere there are hydrophobic interactions, more pronounced for CP17 (Fig. 5d). Interestingly, the greater bulk of CP17 means that the C-terminal residues of VP3, which becomes ordered on GSH binding remains disordered upon binding of CP17. An opportunity for increased affinity might therefore come from utilising specific interactions.

![Graph showing the effect of GSH on PV VLP thermostability.](image)

**Fig. 4** GSH has a stabilising effect on PV VLPs in vitro. Proportion of D antigen reactivity remaining after heating in the presence or absence of 1, 5 and 10 mM GSH. Aliquots of IPV and VLPs were incubated at a range of temperatures and analysed by D Antigen ELISA; reactivity is expressed relative to samples incubated at 4 °C for the same period. Data were shown for PV3-SC8 VLPs expressed in mammalian cells.

**Table 3** Effect of GSH on PV VLP thermostability.

| VLP Prep* | Temperature (°C) at which 50% D-Antigenicity was lost | Temperature difference between treated and non-treated samples |
|-----------|-----------------------------------------------------|-------------------------------------------------------------|
|           | No GSH | +1 mM GSH | +5 mM GSH | +10 mM GSH |
| IPV (type 3) | 50.0 °C | 55.0 °C (13 °C) | >55.0 °C (13 °C) | >70.0 °C (18 °C) |
| MVA PV2 MEFS6b | 52.0 °C | 66.0 °C (110 °C) | 67.0 °C (11 °C) | 67.0 °C (11 °C) |
| MVA PV3 SKtSC8 | 56.0 °C | 60.0 °C (10 °C) | 45.5 °C (7 °C) | 42.5 °C (18 °C) |
| MVA wt PV2 | 34.5 °C | 40.5 °C (16 °C) | 41.5 °C (7 °C) | 41.0 °C (16 °C) |
| MVA wt PV3 | 35.0 °C | 40.5 °C (16 °C) | 41.5 °C (7 °C) | 41.0 °C (16 °C) |
| Yeast PV1 MahSC6b | 43.5 °C | 48.0 °C (145 °C) | 52.5 °C (19 °C) | 51.5 °C (18 °C) |
| Yeast PV2 MEFS6b | 45.5 °C | 48.0 °C (125 °C) | 50.0 °C (145 °C) | 50.0 °C (145 °C) |
| Yeast PV3 SKtSC8 | 55.0 °C | 55.5 °C (10.5 °C) | 53.0 °C (12 °C) | 55.0 °C (=) |

*IPV inactivated polio vaccine (from derived virus).
*Both mammalian-expressed (MVA) and yeast-expressed VLPs had naturally derived pocket factors.
with these terminal residues, although there would be an entropic penalty to their ordering.

It is interesting to note that CP17, which was originally designed to target the lipid-binding pocket within VP1, binds very specifically at a completely separate site and shows no evidence of binding within the targeted pocket; the features there being entirely consistent with the binding of natural lipids in the wt PV2-CP17 structure (Supplementary Fig. 3d) or that of the pocket factors in the PV3-SC8 GPP3+GSH and PV3-SC8 pleconaril+GSH complexes (Fig. 1f and Supplementary Fig. 2f). We note that the binding of CP17 is
reported to be weak (>400 μM) against PV1 and not therefore suitable for use directly as a therapeutic candidate or even for vaccine formulation.

CP48 is reported to be a tighter PV binder than CP17 and has been observed bound to CVB4. We, therefore, attempted to also determine the structure of the complex of CP48 with wt PV2. However, analysis at 2.7 Å resolution provided no evidence of binding (data not shown). We repeated the analysis with a compound derived directly from the research group that had shown binding to CVB4; however, there was no change in the result, suggesting that this molecule does not bind PV2, despite the reported EC₅₀ of 27 nM against PV1.

Discussion

The data presented here, together with previous publications, show that a GSH-binding site is conserved across many enteroviruses. The fact that these are spread across a structure-based phylogenetic tree of enteroviruses supports the hypothesis that there is a unified assembly mechanism common to the majority of enteroviruses (Fig. 3e). The binding of a single GSH molecule locks together two adjacent VP1 subunits within a pentamer and in the process confers order on the C-terminus of the VP3 subunit, providing a mechanism of stabilisation by GSH during capsid morphogenesis and the formation of pentameric assemblies. It should be noted that the GSH-binding pocket is not conserved in the expanded C-antigenic poliovirus structure. In the latter, the pocket is opened by approximately 5 Å so that the key stabilising interactions cannot be made (Supplementary Fig. 4), explaining the observed stabilisation of the D-antigenic state by GSH. GSH, therefore, stabilises only the native D-antigenic state and D→C conversion would be expected to eject both GSH and the pocket factor. Similar conformational changes between two particle states are seen in other GSH-binding enteroviruses, and thus the use of GSH affinity columns for entervoivirus purification will likely select for the D-antigenic states and could lead to loss/removal of material for less stable viruses and VLPs.

In line with previous results, putative antiviral CP17 also binds in the GSH pocket in poliovirus, suggesting that it has a conserved biological function, this site may offer the potential for broad-spectrum inhibition of enteroviruses. Surprisingly, however, we were unable to observe the binding of CP48, despite this compound being reported as having a higher affinity for PV (the reported potencies of CP17 and CP48 against PV1 Mahoney are >400 and 27 μM EC₅₀, respectively). Nevertheless, the high-resolution data we provide for both GSH and CP17 binding inform the pharmacophore definition and optimisation of small molecules to bind more strongly at the GSH pocket than GSH or CP17. Specifically, we suggest that the benzyl portion of the isobenzopyrrolo-1,3-dione moiety of CP17 might be modified to provide groups able to make polar or charge interactions with the carbonyl oxygen of VP1-Ile89 or the carboxylate of the sidechain of VP1 Asp114 (Fig. 5e, g).

In addition to the potential of such compounds as antivirals they (and indeed GSH) may prove useful for the stabilisation of vaccine candidates against enteroviruses, namely VLP-based vaccines such as those we are developing to provide poliovirus vaccines for the post-eradication era. Finally, since the mechanism of action of the GSH-pocket binders and the VP1 lipid-pocket binders is quite separate, they should act additively as either inhibitors or stabilisers, with reduced risk of resistance mutations. It may be interesting to experimentally address such potential synergistic effects, which could be exploited to provide a pathway to the development of new therapeutics against a range of disease-causing enteroviruses.

Methods

Production of PV VLPs using yeast and mammalian expression systems. The production and purification of PV VLPs using yeast and mammalian expression systems has been described elsewhere. For yeast-expressed PV VLPs, the P1 gene of PV3-SC8 and an uncleavable 3CD promoter expression vector was constructed through PCR amplification from position 1 of the 3CD pPink-HC expression vector multiple cloning site (MCS) using EcoRI and FoeI (NEB). Subsequently, a dual promoter expression vector was constructed through PCR amplification from position 1 of the 3CD pPink-HC to position 1285, inserting a Sacl restriction site at both the 5′ and 3′ end of the product. The P1 expression plasmid was linearised by Sacl (NEB), and then the 3CD PCR product inserted. The resulting plasmid was linearised by AfII digestion (NEB) and transformed into PichiaPink strain One (Invitrogen, USA) by electroporation.

Transformed yeast cells were screened for high-expression clones by small-scale expression experiments (5 ml cultures), with levels for each clone determined by immunoblotting. For VLP production, cultures were brought to high density in 200 ml YPD in 2 L baffled flasks. After 24 h, the cultures were pelleted at 1500×g and resuspended in YPM (methanol 0.5% v/v) to induce protein expression and cultured for a further 48 h at 28 °C. Cultures were fed an additional 0.5% v/v methanol at 24 h post-induction. After 48 h, cells were pelleted at 10000g and resuspended in breaking buffer (50 mM sodium phosphate, 5% glycerol, 1 mM EDTA, pH 7.4) and frozen prior to processing.

Cell suspensions were thawed and lysed using a CF-1 cell disruptor at ~275 MPa chilled to 4 °C following the addition of 0.1% Triton-X 100. The resulting lysate was clarified through multiple rounds of centrifugation and a chemical precipitation step as previously described. The clarified supernatants were then concentrated through a 30% sucrose cushion. The resulting pellet was resuspended in PBS + 1% NP40 + 0.5% sodium deoxycholate and centrifuged at 10,000g. The supernatants were then purified through 15–45% sucrose gradients. Gradients were collected in 1 ml fractions from top to bottom and analysed for the presence of VLPs through immunoblotting and ELISA.

Briefly, for mammalian-expressed PV VLPs, PV-specific gene sequences for the P1 region were cloned in frame with a C-terminus T7 promoter and the 3CD promoter from PV3-SC8. Plasmids derived from this plasmid were transformed into modified vaccinia virus Ankara (MVA) transfer vectors upstream of an unclavable 3CD sequence derived from PV1 Mahoney with native sequence. In the resulting
dicistronic cassettes co-expression of 3CD alongside P1 was regulated using a PV3-ARTICLE COMMUNICATIONS BIOLOGY | https://doi.org/10.1038/s42003-022-04252-5

expression cassette included an FMDV IRES upstream of P1, the FMDV 3

cassettes co-expression of 3CD alongside P1 was regulated using a PV3-ARTICLE COMMUNICATIONS BIOLOGY | https://doi.org/10.1038/s42003-022-04252-5

Cryo-EM image processing

Cryo-EM data preparation was similar for all samples. Three to four microliters of VLP-compound or VLP-compound-GSH mixture were applied to glow-discharged Lacey carbon copper grids with an ultra-thin carbon support film (produced at VSEL, Agar Scientific). After 30 s, excess electron-dense material was removed by manual blotting with filter paper. To increase the number of particles in the holes, grids were re-incubated with a further 3 s of sample for 30 s, followed by mechanical blotting for 3–4 s and rapid vitrification in a liquid ethane/propane mixture with a Vitrobot Mark IV plunge-freezing device (Thermo Fisher Scientific). After 4 s and rapid vitrification, the unbound sample was discarded. For PV3-SC8pleconaril and wt PV2-CP17 data were subjected to CTF refinement by using a Fourier shell correlation (FSC) threshold of 0.1433. The maps for each reconstruction were sharpened using Post-processing in RELION by applying inverse B-factors of −52.3, −72.2 and −53.6 Å² for PV3-SC8pleconaril, PV3-SC8pleconaril + GSH and wt PV2-CP17, respectively. The wt PV2-CP17 data were subjected to Ewald sphere correction to further improve the resolution, followed by sharpening with an ad-hoc B-factor of −15.0 Å² after testing a range of values for map interpretability. Local resolution was estimated for each reconstruction using the RELION implementation of local resolution algorithm, and locally scaled maps were used for model building and refinement in all cases. Data processing statistics are summarised in Table 1.

Atomic model building, refinement and analysis

For PV3-SC8pleconaril + GSH, the atomic coordinates of the previously determined structure of PV3-SC8 (PDB ID 5OSB) were manually placed into the cryo-EM electron potential maps using UCSF Chimera. Manual fitting was optimised with the UCSF Chimera ‘Fit in Map’ command and the ‘Rigid Body Fit’ function in Coot. For the wt PV2-CP17 structure, the atomic coordinates of PV2 Lansing strain (PDB ID 1EAH) were used and a similar procedure was applied to optimise the initial fit. For all structures, the cryo-EM map surrounding a single capsid protein (subunits VP0, VP1 and VP3) was extracted using phenix.map_box within Phenix. Manual rebuilding was performed on these models using the tools in Coot, followed by iterative positional and B-factor refinement in real-space using phenix.real_space_refine within Phenix. All refinement steps were performed in the presence of hydrogen atoms. Chemical constraints for GPP3, pleconaril and CP17 were generated using the grade server. Only atomic coordinates were refined; the maps were kept constant. Each round of model optimisation was guided by cross-correlation between the map and the model. Final models were validated using MolProbity, EMRinger23 and CalBAM integrated within Phenix. Refinement statistics are shown in Table 2. Interface analysis of the PV3-SC8pleconaril + GSH, PV3-SC8pleconaril + GSH and wt PV2-CP17 binding pockets was performed using the ‘Protein interfaces, surfaces and assemblies’ service PISA at the European Bioinformatics Institute. (http://www.ebi.ac.uk/pdbe/prot_int/pistart.html)44. Gap-penalty-weighted structural superpositions of capsid proteins were performed with a version of the program SHP modified to estimate the evolutionary distance. A full matrix of evolutionary distances was calculated, and the phylogenetic tree for the variation in distance was generated using the programs FITCH and DRAWTREE, as part of the PHYLIP package. Sequence alignments were generated with Clustal Omega19 and Espirit (https://espiritz.ibcfr.fr)40. Ligand interaction diagrams were prepared using Ligplot+ v.2.21. Molecular graphics were generated using PyMol28 and UCSF Chimera30.

Stability measurements of GSH binding to PV VLP. The temperature at which a conformational change from D to C antigenicity occurred was measured by heating at a range of temperatures from 30–70 °C followed by D antigen ELISA. Samples were diluted in 6-salt PBS with or without GSH to twice the concentration required to obtain an OD of 1.0 in D antigen ELISA, duplicate samples heated for 10 min at each temperature were then diluted 1:1 with 2% dried milk in 6-salt PBS and cooled on ice. D antigen content was measured by a non-competitive sandwich ELISA assay developed to measure the D antigen content of poliovirus vaccines. Briefly, twofold dilutions of antigen were captured with a serum-specific polyclonal antibody, then detected using isotype-specific, D antigen-specific monoclonal antibodies followed by anti-mouse peroxidase conjugate. The monoclonal antibodies used were 234 for type 1, 235 for type 2 and 520 for type 3.

Reporting summary. Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The atomic coordinates for PV3-SC8pleconaril + GSH, PV3-SC8pleconaril + GSH and wt PV2-CP17 have been deposited in the Electron Microscopy Data Bank under accession codes EMD-15725, EMD-15726 and EMD-15727.
References

1. Bailey, E. S., Fieldhouse, J. K., Choi, J. Y. & Gray, G. C. A mini review of the zoonotic threat potential of influenza viruses, coronaviruses, adenoviruses, and enteroviruses. Front. Public Health. 6, 104 (2018).

2. Tuthill, T. J., Groppelli, E., Hogle, J. M. & Rowlands, D. J. Picornaviruses. PLoS Pathog. 8, e1002764 (2012).

3. Basavappa, R. et al. Role and mechanism of the maturation cleavage of VP0 in poliovirus assembly: structure of the empty capsid assembly intermediate at 2.9 Å resolution. Proteins. 76, 1561–1604 (2016).

4. Wang, X. et al. A sensor-adaptor mechanism for enterovirus uncoating from structures of EV71. Nat. Struct. Mol. Biol. 19, 424–429 (2012).

5. Tsang, S. K., Danthi, P., Chow, M. & Hogle, J. M. Stabilization of poliovirus by antiviral agents that inhibit uncoating. Science 233, 1286–1293 (1986).

6. Fox, M. P., Otto, M. J. & McKinlay, M. A. Prevention of rhinovirus and poliovirus uncoating by WIN 51711, a new antiviral drug. Antimicrob. Agents Chemother. 30, 110–116 (1986).

7. Scheres, S. H. & Chen, S. Prevention of overamplification in RELION-3.1. IUCrJ 9, 2253–2267 (2020).

8. Wang, X. et al. A sensor-adaptor mechanism for enterovirus uncoating from structures of EV71. Nat. Struct. Mol. Biol. 19, 424–429 (2012).

9. De Palma, A. M. et al. Potential use of antiviral agents in polio eradication. Emerg. Infect. Dis. 14, 545–551 (2008).

10. Global polio eradication initiative. Polio now (August 2022).

11. Amassari, D. C., Porter, D. C. & Morrow, C. D. Coinfection with recombinant vaccinia viruses expressing poliovirus P1 and P3 proteins results in polyprotein processing and formation of empty capsid structures. J. Virol. 65, 2088–2092 (1991).

12. Ferguson, M., Wood, D. J. & Minor, P. D. Antigenic structure of poliovirus in inactivated vaccines. J. Gen. Virol. 74, 685–690 (1993).

13. Basavappa, R. et al. Role and mechanism of the maturation cleavage of VP0 in poliovirus assembly: structure of the empty capsid assembly intermediate at 2.9 Å resolution. Proteins. 3, 1651–1699 (1994).

14. Fox, H., Knowshon, S., Minor, P. D. & Macadam, A. J. Genetically thermomodulated, immunogenic poliovirus empty capsids; a strategy for non-replicating vaccines. PLoS Pathog. 13, e1006117 (2017).

15. Chait, B. T. & Brown, D. J. Protein mimicry by virus-like particles as a proof of principle for next generation polio vaccines. NPJ Vaccines 6, 5 (2021).

16. Sherry, L. et al. Comparative molecular biology approaches for the production of poliovirus virus-like particles using Pichia pastoris. mSphere https://doi.org/10.1128/mSphere.00838-19 (2020).

17. Xu, Y. et al. Virus-like particle vaccines for poliovirus types 1, 2, and 3 with enhanced thermostability expressed in insect cells. Vaccine 37, 2340–2347 (2019).

18. Farzad, B. et al. MAH719, a novel nanobody-expressing virus-like particles a proof of principle for next generation polio vaccines. PLoS Pathog. 14, e1007620 (2018).

19. Williams, C. J. et al. MoProbyte: more and better reference data for improved all-atom structure validation. Protein Sci. 27, 293–315 (2018).

20. Serra, H. et al. Plant-made polio type 3 stabilized VLPs—a candidate synthetic polio vaccine. Nat. Commun. 8, 9 (2017).

21. Serra, H. et al. Plant-made polio type 3 stabilized VLPs—a candidate synthetic polio vaccine. Nat. Commun. 8, 245 (2017).

22. Sutter, R. W., Modlin, J. F. & Zaffran, M. Complete polio eradication: the case for antiviral drugs. J. Infect. Dis. 215, 333–334 (2017).

23. Collett, M. S., Neys, J. & Modlin, J. F. A case for developing antiviral drugs against polio. Antivir. Res. 79, 179–187 (2008).

24. Mari, M., Morales, A., Colle, A., Garcia-Ruiz, C. & Fernandez-Checa, J. C. Mitochondrial glutathione, a key survival antioxidant. Antioxid. Redox Signal 11, 2685–2700 (2009).

25. Meister, A. & Anderson, M. E. Glutathione. Annu. Rev. Biochem. 52, 711–760 (1983).

26. Cao, Z. et al. Isochirolic acid C prevents enterovirus 71 infection via modulating redox homeostasis of glutathione. Sci. Rep. 7, 16278 (2017).

27. Ma, H. C. et al. An interaction between glutathione and the capsid is required for the morphogenesis of C-cluster enteroviruses. PLoS Pathog. 10, e1004052 (2014).

28. Mikami, T., Satoh, N., Hatayaama, I. & Nakane, A. Buthionine sulfoximine inhibits cytopathic effect and apoptosis induced by infection with human echovirus 9. Arch. Virol. 149, 1117–1128 (2004).

29. Thiibaut, H. J. et al. Binding of glutathione to enterovirus capsids is essential for virion morphogenesis. PLoS Pathog. 10, e1004039 (2014).

30. Smith, A. D. & Dawson, H. Glutathione is required for efficient production of infectious picornavirus virions. Virology 353, 258–267 (2006).

31. Abdellah, R. et al. A novel druggable interprotomer pocket in the capsid of rhino- and enteroviruses. PLoS Pathog. 17, e3000281 (2019).
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Competing interests

The authors declare no competing interests.

Additional information

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Author contributions

Experiments were conceived and designed by M.W.B. and D.I.S. M.W.B., V.N., H.F., L.S. and K.G. performed experiments. M.W.B. and V.N. collected and processed the cryo-EM data, built and refined atomic models and, along with C.P., E.E.F. and D.I.S. analysed cryo-EM results. H.F. performed the GSH thermostability assay and, along with A.J.M. analysed the results. All authors, including N.J.S. and D.J.R. interpreted the results. M.W.B., C.P., H.F., E.E.F. and D.I.S. wrote the manuscript, and all authors reviewed and edited the manuscript.